Surgery for Macular Disease

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Thesis Declaration

I, Gurmit Singh Uppal confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

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Abstract

The MD will primarily examine the role of surgery in the management of the wet form of age related macular degeneration (AMD) and secondarily for specific inherited macular dystrophies. It is postulated that in the early stages of wet AMD and other sub-foveal disorders involving choroidal neovascular membranes (CNV), photoreceptor loss is relatively limited with the disease confined to the sub-foveal layers, namely the choriocapilliaris-Bruch membrane-retinal pigment epithelium (RPE) interface.

At this stage the retina is affected functionally and reversibly but with time the damage becomes permanent and irreversible. As such a critical window of opportunity exists to: 1. Salvage function from the existing photoreceptor pool before fibrovascular proliferation causes marked 'irreversible' photoreceptor loss 2. Treat any visual loss that may be due to secondary and potentially 'reversible' factors such as sub-foveal fluid and haemorrhage and 3. Mechanically restore normal anatomy.

Previous attempts at sub-macular surgery have been associated with the loss of RPE in the area of the CNV during removal that secondarily causes degeneration of photoreceptors. Consequently, different innovative surgical approaches, including 360-degree macular translocation and full thickness autologous RPE transplantation, are under investigation for the management of sub-foveal CNV. The rationale of surgery in both techniques is to effectively restore the choriocapilliaris-Bruch's-RPE interface beneath the foveal photoreceptors and rescue function before fibrovascular proliferation causes marked 'irreversible' photoreceptor loss.

Pilot studies have been established to: 1. Examine the surgical feasibility and the anatomical and functional outcomes for each procedure 2. Investigate the pathophysiology of the underlying disease processes. In addition, a number of parameters will be investigated to evaluate the quality of recovery of vision. This will include assessing fixation stability, reading ability, histopathological studies and electrophysiological correlates.

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I Management of Age Related Macular Degeneration and other Macular Disease

Irreversible central visual loss is the profoundly debilitating end stage of a number of macular diseases. The best documented, age related macular degeneration (AMD), is characterised by degeneration of the retinal pigment epithelium (RPE) and associated secondary loss of the overlying neuroretina (Bressler et al., 1994). The most severe form of the disease, exudative AMD, is further recognised by the development of the sub-foveal choroidal neovascular membrane (CNV) that invariably results in a rapid and marked visual loss (Green, 1999, Bressler et al., 1992). In addition to AMD, CNV also comprises the intrinsic element when considering severe visual loss in pathological myopia, presumed ocular histoplasmosis syndrome (POHS), angiod streaks and a variety of other disorders (Adelberg et al., 1995, Avila et al., 1984, Lim et al., 1993, Thomas et al., 1994, Berger and Kaplan, 1992).

AMD is the leading cause of severe visual impairment and legal blindness in the developing world among those aged over 65 (Vingerling et al., 1995, van Leeuwen et al., 2003a, van Leeuwen et al., 2003b, Mitchell et al., 1995, Attebo et al., 1996, Klein et al., 1992, Cruickshanks et al., 1997, Augood et al., 2006). The disorder is categorised into the non-exudative (dry) form and the exudative (wet) form. The dry form accounts for the majority of cases and 10% of the severe visual loss associated with AMD. This is attributable to geographic atrophy, a confluent loss of choriocapilliaris and RPE with associated loss of overlying photoreceptors. In contrast, 10-20% have the wet form, 90 % of which develop severe visual loss (Snellen visual acuity 20/200 after two years) secondary to CNV. CNV is an in growth of friable fenestrated new vessels from the choriocapilliaris, through Bruch's membrane, and into the RPE and neurosensory retina. This results in the mechanical disruption of local anatomy, fluid and lipid leakage, and haemorrhage. The net effect is the loss of RPE, photoreceptors and eventual fibrous scarring. Over the next 20 years as worldwide demographics shift to the right, the prevalence of AMD is estimated to grow by 50% with major socio-economic implications (Wang et al., 1999b, Wang et al., 1999a, Bressler et al., 2003, Friedman et al., 2004).

In addition to disorders involving CNV, there are a number of inherited macular dystrophies, which result in progressive bilateral macular degeneration, and permanent loss of central vision (Michaelides et al., 2003, Michaelides et al., 2005, Francis et al., 2005). Despite a wide phenotypical variation in these disorders, a common observation, as with AMD, is RPE degeneration accompanying the neuroretinal changes. However unlike AMD, it is uncertain whether the RPE changes are a secondary response to primary neuroretinal dysfunction, or whether RPE driven dysfunction results in neuroretinal degeneration.

The purpose of interventions for wet AMD and other macular diseases is to significantly alter the natural history of visual loss, particularly that associated with sub-foveal CNV. The last few decades have been dominated initially by vaso-destructive, and more recently by pharmacological therapies, directed against the final stages of the disease pathway – the CNV. A greater understanding of the role of the bio-molecular processes involved in normal retinal physiology and in the pathogenesis of retinal disease, together with the central role of the RPE in these pathways, has seen the emergence of cell based therapies and the concept of cell replacement with 'healthy' RPE.

While significant advances have been made with pharmacological agents such as vascular endothelial growth factor (VEGF) inhibitors, there remains no treatment for advanced wet AMD where there is anatomical disruption of the retina, and there are presently no treatments available for the macular dystrophies. The challenge therefore is to offer cell based therapeutic interventions that not only restore structure and recover lost function but also offer a potential cure with improved vision that is both stable and longstanding. As such the development of advanced surgical techniques has resulted in a number of procedures, namely macular translocation and RPE transplantation that provide the real prospect of meeting these challenges.

Therapeutic Approaches

Strategies to treat AMD are primarily directed at the advanced stages of the disease only. Other than the small beneficial effects of vitamin and mineral supplementation (2001b), there is no proven therapy that prevents the development of AMD, and in particular CNV. As such numerous interventions have been suggested for CNV including pharmacological, radiation, photocoagulation, photodynamic and surgical therapies. To date the only proven treatments (level four evidence) to have a beneficial effect on the natural history of AMD are photocoagulation, photodynamic therapy with verteporfin dye injection (PDT) and more recently the anti-VEGF agents (Pegaptanib (Macugen™), Ranibizumab (Lucentis™), Avastin™ (bevacizumab)).

Laser photocoagulation stabilises vision in sub-foveal lesions, however the treatment is associated with an immediate and often unacceptable loss of central vision due to irreversible direct damage to the retina (Group, 1991a, Group, 1991b, Group, 1994). PDT typically leads to CNV closure after treatment, but reperfusion commonly occurs after 3 months and re-treatments are often necessary. PDT is characterised by a loss of 15 letters of vision from baseline over the course of treatment, requiring an average of 5.5 treatments over two years (Group, 1999, Group, 2001a, Group, 2001b). Both photocoagulation and PDT are beneficial in only a limited number of patients with CNV, and their main benefit is to lessen the risk of severe and moderate vision loss respectively in patients with sub-foveal CNV rather than reverse it. The current generation of anti-VEGF agents have shown more far more promise in regressing CNV and improving vision. Macugen™ was the first anti-VEGF treatment introduced and although superior to PDT in that it is effective in all types of neovascular AMD, it too was associated with a loss of vision over the course of treatment (broadly similar to PDT results) (Gragoudas et al., 2004). In contrast several recent studies support the use of both Lucentis™ and Avastin™ both of which are associated with an average improvement in vision ranging from 4.6 to 11 letters (Rosenfeld et al., 2006, Brown et al., 2006, Heier et al., 2006, Regillo DC, 2008, Avery et al., 2006, Spaide et al., 2006). The reported outcomes require regular re-treatments and the long-term efficacy of these agents is yet to be determined. Additionally, as the pathogenic theory of AMD is unraveled (see below), it is clear that the disease is a complex interplay of genetic susceptibility and environmental factors leading to the development of pathological oxidative and inflammatory processes occurring in the face of constant physiological cellular stress at the level of

the RPE. As such it is questionable if a single treatment targeted at one component of a vaso-inflammatory pathway can be considered to be curative. Furthermore, the anti-VEGF agents are unsuitable in the presence of significant sub-macular haemorrhage and or the gross loss of normal retinal architecture secondary to the disease (pigment epithelial detachments; RPE rips). Other non-surgical treatments such as indocyanine green (ICG) guided treatment of feeder vessels, interferon, radiation, and the anti-angiogenic role of steroids have proved to be ineffective or unproven in the treatment of sub-foveal CNV (Poliner LS, 1993, Desatnik et al., 2000, Marcus et al., 2004, Hart et al., 2002, Geltzer et al., 2007).

The increasing prevalence of AMD, combined with the absence of a treatment modality resulting in an improvement in visual function and suitable for all lesion types prompted a search for alternative surgical therapies. A variety of surgical approaches have been developed for advanced AMD lesions including the pneumatic displacement of sub-macular blood and surgical removal of CNV. The former removes the cause of the immediate scotoma impairing vision however this treatment does not address the underlying pathology. In contrast, surgical removal of the CNV, alone, has not resulted in improved function (Bressler et al., 2000, Hawkins et al., 2004b, Bressler et al., 2004) and has only been shown to be of small benefit in patients with POHS with acuities of 20/100 or worse (Hawkins et al., 2004a). The poor results with this form of sub-macular surgery are attributed to the obligatory loss of RPE and underlying choriocapilliaris in the area of the CNV during removal of the membrane complex. It is the loss of the choriocapilliaris-Bruch's-RPE axis that results in secondary degeneration of the overlying photoreceptors, consequently, different innovative surgical approaches, including macular translocation and RPE transplantation, have developed for the management of sub-foveal CNV. In the early stages of wet AMD, and other sub-foveal disorders, photoreceptor loss is relatively limited and visual loss may be secondary to the loss of retinal architecture from potentially 'reversible' factors such as sub-foveal fluid and haemorrhage. The rationale for surgical intervention with macular translocation and RPE transplantation is, following removal of the CNV, to effectively restore the choriocapilliaris-Bruch's-RPE interface beneath the foveal photoreceptors before CNV associated fibrovascular proliferation causes marked and 'irreversible' photoreceptor loss.

In order to place foveal photoreceptors on an area of healthy choriocapilliaris-Bruch's-RPE two methods are presented. Firstly, moving the foveal photoreceptors

to an area of healthy underlying RPE (macular translocation) and secondly moving healthy choriocapilliaris-Bruch's-RPE and placing it under the foveal photoreceptors (RPE transplantation). Macular translocation can be achieved by inducing a retinal detachment followed by techniques involving incisions of the retina and direct retinal manipulation ('full macular translocation') or a chorioretinal shortening procedure ('limited macular translocation') in order to reposition the neurosensory retina over healthy tissue. RPE transplantation, by contrast, aims to remove and replace the diseased sub-foveal layers. This involves the removal of the CNV through a small retinotomy followed by replacement of the RPE ('partial thickness transplantation') or the choriocapilliaris-Bruch's-RPE complex ('full thickness' transplantation) with autologous tissue. Both approaches re-align viable photoreceptors with healthy supporting tissue and provide the opportunity to treat the CNV either directly through removal or indirectly by the creation of an extra-foveal CNV location accessible to established non-surgical treatments.

This thesis will examine these two surgical approaches used to treat sub-foveal macular disease, primarily in wet AMD, but also for an inherited macular dystrophy. Macular surgery aims to directly treat the pathology, restore normal retinal anatomy and function, as well as treating the secondary sight threatening complications (sub-foveal fluid and haemorrhage). The aims are to examine the feasibility of the techniques, the anatomical restoration of foveal anatomy, and the functional success in terms of quantitative and qualitative recovery of vision. The thesis will also consider prospective applications of these techniques that may extend beyond the treatment of sub-foveal CNV with recent visual loss, to patients with early or dry macular degeneration, patients with macular dystrophies with associated sub-foveal dysfunction, the use of artificial RPE membranes, and the combined use of cell and gene replacement.

II Background

A Sub-Retinal Anatomy

The retina is the image plane of the eye's optical system and is the innermost of the three coats of the eye. It consists of two primary layers that are derived from the embryological inner and outer layers of the invaginated optic cup. These are, an inner *neurosensory retina* and an outer simple epithelium, the *retinal pigment epithelium*. The layers anatomically extend from their anterior termination at the ora serrata to the optic nerve head. Each layer is bound by its respective basal lamina, also derived from the embryonic optic cup. Thus, the RPE is bound on its external aspect by Bruch's membrane and the neurosensory retina is bound at its internal aspect by the internal limiting membrane. The two layers adhere across a potential space that exists between them, known as the *sub-retinal space*.

The neurosensory retina is the thin transparent layer of highly organised and metabolically active neural tissue where light stimuli are converted by photoreceptors (rods and cones) to neural impulses. The neural impulses are partially locally integrated before transmission to the occipital cortex via the ganglion cells in the optic nerve. The photoreceptors are located on the outer aspect of the neurosensory retina where their outer segments interdigitate with RPE cells. Photoreceptor density varies with location with the peripheral retina being rod dominated whilst the density of cones increases near the macula with the fovea consisting exclusively of cones. The neurosensory retina receives a dual blood supply, the inner two thirds is supplied via the retinal circulation and the outer third is supplied from the choroidal circulation via the choriocapilliaris and RPE. At the fovea, the inner layers of the neurosensory retina are laterally displaced to provide direct access to light to the outer layers consisting purely of cones. The foveal neurosensory retina is avascular and relies solely upon the choriocapilliaris for support.

The RPE is a continuous monolayer of hexagonally arranged epithelial cells that vary in size and shape depending on age and location. The basal aspect of the cells rest on Bruch's membrane and their apical surfaces are characterised by apical microvilli that are intimately associated with photoreceptor outer segments. RPE cells have a low regenerative capacity and cell loss is accommodated by hyperplasia of adjacent cells resulting in loss of the regular hexagonal array, a typical finding in older eyes. The RPE performs a number of physical and biochemical functions that sub-serve local cellular and extracellular homeostasis and are pivotal to optimising photoreceptor function. Through the expression and activity of specific proteins the RPE maintains adhesion between the RPE layer and the neurosensory retina, synthesizes inter-photoreceptor matrix, forms a selectively permeable barrier between the choroid and neurosensory retina (part of the blood-retinal barrier), contributes to photoreceptor renewal by phagocytosing and degrading the spent tips of outer segments, absorbs excessive high-energy light and reduces light scatter within the eye, protects against light-generated reactive oxygen species, and aids the transport and storage of metabolites and vitamins. The RPE has also been shown to exert considerable trophic functions on the surrounding cellular matrix, choriocapilliaris, and neurosensory retina through the secretion of vasoactive factors such as pigment epithelial derived factor (PEDF) and VEGF. These factors are known to be critical to the pathways of inflammation and neovascularisation, both of which are implicated in the pathogenesis of AMD. Thus, the RPE layer occupies a central position, together with photoreceptors, effectively working together to facilitate transduction of the light stimulus and provide vision.

The RPE rests on Bruch's membrane, a modified connective tissue layer. Bruch's membrane comprises five layers from the innermost RPE basal lamina, an inner collagenous zone, a middle elastic layer, an outer collagenous zone and the outermost basement membrane of the endothelial cells of the choriocapilliaris. Bruch's membrane is located such that it behaves as a two-way conduit for nutrients and cellular breakdown products between the metabolically active RPE and the choriocapilliaris. The outermost component of what is considered sub-retinal anatomy, before the middle coat of the eye is encountered, is the choriocapilliaris. This is a rich network of capillaries that are fenestrated on their inner or retinal aspect and are also arranged in a hexagonal manner. As mentioned the choriocapilliaris provides a blood supply to the outer third of the neurosensory retina and an exclusive supply to the foveal photoreceptors. The choroidal circulation that forms the middle

vascular coat of the eye feeds the choriocapilliaris.

B Clinico-Pathological Forms of Age Related Macular Degeneration

Age related maculopathy (ARM) is the precursor to AMD and is characterised by degenerative changes occurring in the central macular area. The earliest pathological changes involve the focal and diffuse accumulation of periodic acid-Schiff positive mebrano-granular and vesicular material in the inner layers of Bruch's membrane (basal linear deposits – BlinD) and between the plasma membrane and basal lamina of the RPE (basal laminar deposit – BlamD) (Green, 1999). These early basal deposits are not routinely visualised on clinical examination and may only be seen in the late phases of angiography. With further accumulation of this material, the clinical hallmark of early AMD is seen in the form of the appearance of drusen. Drusen represent an accumulation of BlinD or a localised detachment of BlamD. They are histologically classified as hard (pinpoint discrete yellow-white lesions; <63μm) or soft (indistinct lesions, tendency to become confluent, calcified or filled with cholesterol, >63μm). In ARM, these accumulations do not account for significant visual loss but are known to affect macular function such as contrast sensitivity and spatiotemporal sensitivity (Sunness JS, 1988, Frennesson C, 1995, Midena E, 1997, Midena E, 1994).

In the presence of pathological deposits lying between the RPE and Bruch's membrane, the progression to AMD is recognised by atrophy of the underlying choriocapilliaris (narrowing of vessel lumens and a reduction in the density of the vascular net) and of the overlying RPE (Bressler et al., 1994). Photoreceptors are metabolically dependent on RPE cells and marked apoptosis of the outer and inner nuclear layers of the neurosensory retina are observed with RPE atrophy (Dunaief et al., 2002). It is these secondary changes that occur with the disruption of the choriocapilliaris-Bruch's-RPE-photoreceptor axis, which account for the visual loss in AMD. At this stage four main morphological forms of AMD can be identified – drusen and focal RPE pigmentary changes, geographic atrophy, RPE detachments, CNV and retinal angiomatous proliferation (RAP). Based on these clinical and morphological patterns, AMD is classified into a non-exudative or dry form (drusen and RPE pigment changes; geographic atrophy) and an exudative or wet form (RPE detachments; CNV and RAP lesions).

In the dry form of the disease, the histological changes outlined above result in the formation of drusen as well as focal detachments, hypo-pigmentation, and atrophy of the RPE. Within areas of RPE loss focal hyper-pigmentation may also occur and this corresponds to hypertrophy of RPE cells with clusters of pigmented cells present in the sub-retinal space and outer neurosensory retina. The visual loss in dry AMD is thought to occur due to impedance to the passage of nutrients and metabolic products between the choroid and the RPE, resulting in RPE atrophy and eventual photoreceptor loss. Geographic atrophy represents a form of dry disease characterised by confluent areas (>175μm) of RPE cell death with accompanying loss of overlying photoreceptors. It commonly occurs following the fading of drusen, in areas of RPE pigmentary disturbance, or after the resolution of an area of RPE detachment or CNV involution. Clinically, well-demarcated irregular patches of RPE and choriocapilliaris loss revealing the underlying choroidal blood vessels are observed. These changes are preceded by diffuse areas of increased auto-fluorescence suggesting that excessive accumulation of RPE lipofuscin is associated with the pathogenesis of geographic atrophy (Holz et al., 2001, Einbock et al., 2005, Holz et al., 2007).

In the wet form of the disease, choroidal vessels proliferate and pass through the thickened Bruch's membrane towards the neurosensory retina. The new vessel complex or CNV may remain in a sub-RPE position, enter the RPE layer, or penetrate the RPE to enter the sub-retinal space. In this way CNV may cause detachments of the RPE, pigment modeling and RPE tears, and detachments of the neurosensory retina. The mechanical and metabolic disruption caused by the CNV to the choriocapilliaris-Bruch's-RPE-photoreceptor axis is further exacerbated by the accumulation of extra-cellular fluid, lipid exudates and haemorrhage from the proliferating vessels. This all occurs on a background of dry pathological changes, resulting in an acute loss of vision as compared to the more chronic loss in the dry form of AMD. The end stage of this process of vascularisation is cell death and reactive gliosis precipitating the formation of an organised disciform scar and permanent visual loss (Bressler et al., 1992). Clinically, CNV appear as green-grey sub-retinal lesions and may be associated with sub-retinal and retinal haemorrhage, fluid or lipid. On angiography CNV lesions leak fluorescein and have been classified as either classic (discrete lesions that hyper-fluoresce early and uniformly with increasing intensity and extent) or occult (diffuse irregular fibrovascular RPE

detachments that hyper-fluoresce late and in a stippled manner or areas of late leakage of undetermined origin).

RPE detachments are associated with soft drusen coalescing to form overlying serous detachments of the RPE and with exudation from sub-RPE CNV (Lafaut et al., 2001). Clinically RPE detachments are observed as uniform elevated lesions that on angiography have well demarcated borders and fill uniformly with increasing intensity. Large and elevated RPE detachments can induce significant tangential stress on the RPE leading to rupture. RPE rupture can be clearly observed as linear breaks with the RPE recoiling under the neurosensory retina, due to its elastic properties, to leave large areas of photoreceptors devoid of RPE support. Vision loss in RPE detachment and rupture occurs due to the mechanical disruption of the choriocapilliaris-Bruch's-RPE-photoreceptor axis resulting in metabolic failure of the RPE or a complete absence of RPE photoreceptor support.

RAP lesions are identified as a unique variant of wet AMD that represent new vessel formation extending from the neurosensory retina to the choroid. In contrast to CNV, RAP lesions are thought to originate as capillary proliferation in the neurosensory retina, extending into the sub-retinal space to either terminate as CNV lesions or to form retinal-choroidal anastomosis (Yannuzzi et al., 2001, Lafaut et al., 2000). There is however a lack of sequential histological evidence to confirm the intra-retinal versus choroidal origin of these lesions and recent studies suggest a choroidal origin for the neovascular complex with the early formation of a retinal choroidal anastomosis without evidence of underlying occult neovascularisation (Freund et al., 2008, Yannuzzi et al., 2008). Clinically these vessels are associated with telangectasia, micro-aneurysms and intra-retinal haemorrhages in the early and intermediate stages, and vascularised RPE detachments in the late stages.

C Pathogenic Theory of Age Related Macular Degeneration

AMD is a degenerative disorder of the choriocapilliaris-Bruch's-RPE-photoreceptor axis at the central macular area. The hallmark of the disease is the focal and diffuse thickening of Bruch's membrane. In the dry disease this is associated with dysfunction, atrophy and loss of the choriocapilliaris and RPE, and in the wet disease the development of CNV. While the clinical features of the disease are well documented, the exact aetiology, the primary locus of the insult, and the pathogenesis remain in dispute. While certain macular diseases have been attributed to specific gene defects, e.g. Leber's congenital amarousis and the gene RPE65 (Acland et al., 2001, Narfstrom et al., 2003), the majority, including AMD, are a complex interplay of several genes and an accumulation of environmental insults.

The Bruch's-RPE-Photoreceptor Axis

Visual function is dependent upon an intact and healthy Bruch's-RPE-photoreceptor axis and the progressive disruption of this arrangement is observed from the very early to the late stages of AMD. In ARM, thickening of Bruch's membrane is clearly observed and it has been postulated that this impedes the flow of nutrients to and metabolites away from the RPE and photoreceptors. Under these circumstances RPE and photoreceptors have to function under sub-optimal conditions and are placed under ever increasing stress. It is possible to envisage how such a scenario could result in subsequent disease progression. The stimulus to these early changes remains unknown. Foveal photoreceptors are implicated, as the disease is predominately localised to the central macular region of the retina. Much attention has also been centred on the RPE, as it is considered pivotal for maintaining optimal photoreceptor structure and function (Marshall, 1987, Boulton and Dayhaw-Barker, 2001).

RPE is a highly metabolically active tissue that is subject to an array of direct environmental insults (light exposure, smoking, oxidative stress). RPE is placed under significant metabolic stress from the repeated removal and turnover of photoreceptor outer segments. This function results in the progressive cellular accumulation of a number of oxidised and phototoxic products, such as the lipid-

protein aggregate lipofuscin, throughout life. The combination of genetic susceptibility and cumulative environmental damage to the RPE is thought to lead to the abnormalities observed in the choriocapilliaris-Bruch's-RPE-photoreceptor interface in AMD.

The RPE is a post-mitotic structure that varies in morphology, density and function across the retina (Panda-Jonas et al., 1996). With increasing age the RPE undergoes a number of irreversible structural and functional changes, particularly at the posterior pole. These include changes in RPE morphology (loss of cell shape, RPE multi-layering, atrophy, reduction in cell density and secondary increase in cell diameter), RPE pigmentation (increase in lipofuscin, hyper-pigmentation, decrease in melanosomes), accumulation of advanced glycation end products, reduction in RPE antioxidant systems, and molecular damage to RPE cells (mitochondrial/DNA damage, protein cross-linking). These changes are known to compromise RPE function and lead to pathological damage requiring intervention.

Role of Oxidative Stress

Repeated and prolonged exposure to oxidants is a feature of a number of chronic age-related diseases including atherosclerosis, neoplasia, and AMD (Winkler et al., 1999, Beatty et al., 2000). The retina is particularly susceptible due to the elevated levels of polyunsaturated fats, high oxygen demand, and chronic light exposure of the photoreceptors and RPE. Oxidative stress occurs when the normal physiological production of reactive oxygen species (superoxide anion, hydroxyl radical, hydrogen peroxide) shifts towards the oxidative components. In this situation, the excess oxygen species impair cellular function by disrupting lipid membranes, surface proteins and DNA. This is particularly apparent in the RPE, which plays a pivotal role in photoreceptor homeostasis by the repeated removal and turnover of photoreceptor outer segments. The outer segments are endocytosed on the apical side of the RPE, processed via phagolysosomes, and the degraded material exocytosed on the basal side. Thus RPE cells are placed under constant and significant metabolic stress. The accumulation of oxidised outer segments inhibits the function of the phagolysosomes (Kaemmerer et al., 2007) and results in the progressive cellular accumulation of a number of oxidised and phototoxic products, such as the lipid-protein aggregate

lipofuscin, throughout life. These changes have demonstrated in the macular region of ageing eyes and those with AMD (Suzuki et al., 2007).

Recently, the candidate gene approach has also yielded the identification of a locus on chromosome 10 containing three tightly linked genes associated with AMD. Of these genes, the age-related maculopathy2 (ARMS2) gene mutation results in reduced levels of ARMS2 mRNA, which codes for a protein that appears to have a functional role in mitochondrial homeostasis (Fritsche et al., 2008). Mitochondrial dysfunction is known to be associated with abnormalities in cellular energy supply, generation of reactive oxygen species, and in the initiation of the apoptotic process. These are all concepts that have been associated with the pathogenesis of AMD. Given the extremely high-energy demands of retinal tissue, the ARMS2 mutation serves to provide a link between a primary genetic mutation and degeneration of the retina.

A number of factors affect the oxidative state of the macular region including protective antioxidant mechanisms such as the presence of the macular pigments: leutin (fovea) and zeaxanthine (para-fovea). These pigments absorb light at 460nm and so reduce the exposure of the photoreceptors to blue light phototoxicity. Thus reduced levels of macular pigment may contribute to the pathogenesis of AMD.

Other antioxidant mechanisms include the presence of the cellular enzymes superoxide dismutase, mitochondrial manganese superoxide dismutase, and copper-zinc superoxide dismutase. These enzyme systems function to compartmentalise and convert oxygen species into less harmful entities. A number of other molecules (ß-carotene, vitamins C and E) have a similar role and operate by scavenging oxygen species. These mechanisms have formed the basis of the AREDS formulation of dietary antioxidant supplementation that have demonstrated marked reductions in the progression of certain subtypes of AMD with modest reductions in the overall progression of AMD (2001b).

Several studies have also clearly established smoking as a risk factor for AMD (Smith et al., 1996, Christen et al., 1996, Vingerling et al., 1996, van Leeuwen et al.,

2003a, Khan et al., 2006b, Chakravarthy et al., 2007). Smoking is a pro-inflammatory activity and so in relation to retinal oxidative stress, promotes the formation of reactive oxygen species. Nicotine has been shown to activate retinal phopholipase A2, an enzyme that triggers the arachadonic acid inflammatory cascade (Sastry and Hemontolor, 1998) and tar contains the pro-oxidant hydroquinone. Hydroquinone has also been shown to reduce extracellular matrix turnover and result in sub-RPE deposits in a murine model (Marin-Castano et al., 2006). Smoking is also associated with lower levels of macular pigments (Nolan et al., 2007).

Role of Inflammation

The role of inflammation and the immune system has been closely implicated in the pathogenesis of AMD from the thickening of Bruch's membrane, to the formation of drusen, and the development of CNV (Johnson et al., 2001, Hageman et al., 2001, Sivaprasad and Chong, 2006). Recent studies have confirmed a strong genetic component to AMD, with a genetic defect identified in up to three quarters of cases with several genes identified as having a large effect (Lotery and Trump, 2007). Using a 'candidate gene' approach, a number of studies have confirmed an association between inflammatory pathways, the immune system, and the risk of AMD. The best-documented example is the complement factor H (CFH) gene mutation on chromosome 1, identifying a Tyrosine – Histidine substitution at amino acid 402 (Y402H Polymorphism) (Edwards et al., 2005, Hageman et al., 2005, Klein et al., 2005).

The complement system constitutes part of the innate immune defence and consists of up to thirty serum proteins that are activated by a classic (antibody binding stimulus) or alternate (pathogenic stimulus/cellular debris) pathway. Each pathway converges to produce activated C3 products that result in anaphylaxis (C3a), cell opsonisation that further enhances antibody binding and phagocytosis (C3b), and eventual target cell lysis through formation of porous membrane channels (membrane attack complex).

CFH is a protein that regulates the activation of the complement cascade. Within the retina, CFH is produced by RPE cells and has also been localised to the choroid, Bruch's membrane and the inter-photoreceptor matrix (Chen et al., 2007). CFH

functions to inhibit the activation of C3 and protects membranes against damage by activated complement. Using a CFH deficient homozygote murine model, the complete absence of CFH has been shown to result in the thinning of Bruch's membrane, accumulation of complement C3 in the retina, altered RPE organelle physiology, and disorganization of photoreceptor end segments (Coffey et al., 2007). In population studies of AMD, the presence of the Y402H polymorphism allele in the gene encoding CFH is associated with a 2.7 fold increase in the risk of AMD (also accounting for up to 50% of the attributable risk of AMD), and this increases to a 7.4 in homozygotes (Edwards et al., 2005, Hageman et al., 2005, Klein et al., 2005). When considering the effect of smoking, the Y402H polymorphism confers a relative risk for late AMD of 34.0 as compared to non-smokers without this polymorphism (Despriet et al., 2006). Furthermore, chronic exposure of RPE cells to oxidised photoreceptor segments down regulates the production of CFH, as do the pro-inflammatory cytokines tumour necrosis factor-alpha and interleukin-6 (Chen et al., 2007).

Other complement factors implicated in AMD include complement component 2 (C2) and factor B (BF). As with CFH, these factors have been associated with conferring a degree of protection, with their absence predisposing individuals to AMD (Gold et al., 2006).

The exact role of complement and the Y402H polymorphism in the development of AMD remains undetermined. Clearly there are both genetic and environmental stimulators of the complement cascade which operate both at a local and systemic. Systemic activation of complement may combine with an altered response of the RPE to cellular injury, suggesting that AMD may be considered a systemic disease with the retina behaving as a vulnerable end organ. In this respect a number of studies have demonstrated elevated levels of systemic inflammatory biomarkers, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in AMD (Seddon et al., 2005). In addition, AMD has been shown to share common risk factors with atherosclerosis and Bruch's membrane has been compared to a specialised vessel wall, with the macular region being a particularly vulnerable site (Sivaprasad et al., 2005). Conversely, local dysregulation of the complement cascade within Bruch's membrane and the RPE may establish a situation where, in

the face of prolonged physiological stress, modified by pathological oxidative processes, chronic local inflammation contributes to disease progression.

Role of Hypoxia and Angiogenesis

Exudative AMD is characterised by CNV formation, to which angiogenesis is integral. Chronic hypoxia and inflammation have both been postulated as mechanisms for initiating the choroidal neovascular response, although a close interplay of both processes is the most probable scenario. Under conditions where there is thickening of Bruch's membrane, together with the associated dysfunction and loss of the choriocapilliaris and RPE, localized hypoxia can be easily envisaged. It is well established that the final common pathway in angiogenesis is driven by VEGF and this molecule is produced in response to hypoxia associated with the RPE, the neurosensory retina and CNV (Gottsch et al., 1990, Shima et al., 1995). Inhibition of this molecule has been used to considerable therapeutic effect in exudative AMD (Gragoudas et al., 2004, Rosenfeld et al., 2006, Brown et al., 2006, Heier et al., 2006, Regillo DC, 2008). While currents medical agents are antibodies or aptamers directed against VEGF to prevent interaction with the VEGF receptor, alternate therapies under investigation include interfering with the expression of VEGF mRNA, VEGF traps (synthetic molecules with a high binding affinity for VEGF), and agents directed against signal transduction post-VEGF receptor activation.

Environmental Factors

Environmental factors are also important in the development of AMD, with smoking (Christen et al., 1996, Smith et al., 1996, Vingerling et al., 1996, Khan et al., 2006b, Chakravarthy et al., 2007), obesity (Cho et al., 2001) and a poor diet (2001a) identified as significant risk factors for the disease. As mentioned atherosclerosis and AMD share common risk factors, and the roles of smoking and diet have been discussed above with respect to oxidative stress and inflammation. The exact role of dietary fats is still unclear and is the subject of current investigations including the AREDS II study. Chronic light exposure has also been suggested as a risk factor for AMD (Mitchell et al., 1998, Khan et al., 2006a) and is known to increase the risk of cataract formation. Cataracts may confer natural protection to AMD, however with

progress in cataract surgery, it remains to be seen whether this intervention will add to the threat of AMD, especially in at risk groups (Margrain et al., 2004).

D Natural History of Neovascular Age Related Macular Degeneration

A number of studies have investigated the natural history of neovascular AMD in order to establish the relationship between disease presentation and progression and visual loss. An examination of natural history also provides valuable information on the influence of disease subtype, the optimal timing for intervention, a risk-benefit analysis for different treatment modalities, and the efficacy of new treatments.

The macular photocoagulation study (MPS) represented the first randomised clinical trial for the evaluation of treatments for AMD. MPS data revealed that over 5 years the majority of eyes lost vision if untreated. Severe visual loss (loss of 6 lines or more of acuity) occurred in 65% of eyes with an initial acuity of 20/100 and a sub-foveal CNV. In terms of treatment, MPS demonstrated favourable outcomes with thermal laser photocoagulation only in a small proportion of eyes with small well de-lineated (classic) extra-foveal and juxta-foveal lesions. Eyes with sub-foveal CNV suffered severe and immediate vision loss secondary to the direct effect of the treatment modality (Group, 1991a, Group, 1991b, Group, 1994).

The next advance in treatment for neovascular AMD was PDT and the subsequent Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) study examined both the efficacy of the treatment as well as the natural history of AMD. The introduction of PDT for the treatment of neovascular AMD resulted in the classification of CNV based on angiographic appearances as either classic/predominantly classic (40%) or minimally classic/occult (60%). The TAP study found the benefits of PDT (a loss of 15 letters or less from baseline) were limited to classic CNV subtypes. The control arm of the TAP study further eluded to the natural history of neovascular AMD and demonstrated that after 2 years severe visual loss occurred in 36% of eyes and 67.5% of eyes had a vision of 20/200 or worse (Group, 1999, Group, 2001a, Group, 2001b).

The present generation of anti-VEGF agents appear to be effective across the various angiographic subtypes of CNV. The first such agent, Macugen™, was found in the VEGF Inhibition in Ocular Neovascularisation (VISION) trials to be broadly similar in outcome to PDT. The control arm of the VISION study further corroborated

the findings of previous studies and reported a rapid 3.5 line loss of vision at 12 months of follow up with 55% of eyes having a vision of 20/200 or worse (Chakravarthy et al., 2006, Gragoudas et al., 2004). The anti VEGF Lucentis™ superseded treatment with Macugen™ as it represented the first intervention to offer improved vision as an endpoint rather than stabilisation or limitation of the degree of visual loss. The natural history arm of the first trial to report outcomes with Lucentis™, the Minimally classic/occult trial of the Anti-VEGF antibody Ranibizumab In the treatment of Neovascular AMD (MARINA) study reported a 2.5 line loss of vision at 12 months with severe visual loss occurring in 14.3% of eyes and 42.9% of eyes having a vision of 20/200 or worse. Similarly at 2 years the MARINA study reported severe visual loss in 22.7% of eyes in the control group with 47.9% of eyes having a vision of 20/200 or worse (Rosenfeld et al., 2006).

The studies of the untreated natural history of vision in neovascular AMD (Table 1) strongly suggest that following the onset of disease there is a rapid progression to severe loss of central vision. Most recently a meta analysis of studies of the natural history and prognosis of neovascular AMD reported an average loss of between 1 and 3 lines of acuity at 3 months, 3 and 4 lines at 12 months and an increasing proportion losing more than 6 lines over time (Wong et al., 2008). Although the analysis found few studies to extend beyond 2 years, it further confirmed that if untreated the prognosis in neovascular AMD is very poor.

Table 1 Natural History for Neovascular AMD

	Treatment Modality			
	Thermal Laser	PDT	Anti-VEGF	
			Macugen™	Lucentis™
Study	MPS *	TAP **	VISION **	MARINA **
Year	1994	2001	2004	2006
Severe Loss of Vision †	65 %	36.0 %	NR	22.7 %
Visual Acuity 20/200 or Worse	NR	67.5 %	55.0 %	47.9 %

PDT	Photodynamic Therapy
VEGF	Vascular Endothelial Growth Factor
MPS	The Macular Photocoagulation Study (Group, 1994, Group, 1991b, Group, 1991a)
TAP	Treatment of AMD with Photodynamic Therapy (Group, 2001a, Group, 1999)
VISION	VEGF Inhibition in Ocular Neovascularisation (Chakravarthy et al., 2006)
MARINA	Minimally classic/occult trial of the Anti-VEGF antibody Ranibizumab In the treatment of Neovascular AMD (Rosenfeld et al., 2006)
NR	Data Not Reported
*	5-Year Follow Up
**	2-Year Follow Up
†	Greater Than 6 Lines Lost on Visual Acuity Chart

III Surgical Approaches

A Surgical Principles

The rationale for macular surgery is to treat sub-foveal pathology and restore the retinal anatomy to a physiological state. Ideally any intervention must occur prior to the onset of significant irreversible photoreceptor loss and with minimal collateral surgical trauma. As previously described, the pathogenesis of CNV and the associated changes (fluid, exudates, haemorrhage, fibrosis) are intimately associated with the sub-retinal structures. Therefore the physical removal of a sub-retinal lesion invariably results in the creation of a defect in the choriocapilliaris-Bruch's-RPE axis (Thomas et al., 1992, Grossniklaus and Gass, 1998, Grossniklaus and Green, 1998). Intuitively then to recover function will necessitate a return to a more normal retinal structure. Several approaches have been undertaken and these can broadly be classified into sub-macular surgery for the removal of haemorrhage and CNV, macular translocation, and RPE transplantation.

Sub-macular surgery primarily treats only the sub-foveal lesion and no attempt is made to reinstate the retinal architecture. This approach requires limited access to the sub-retinal space to directly remove CNV or haemorrhage. In contrast, the primary aim of macular translocation and RPE transplantation is to not only directly treat CNV but also to re-establish the choriocapilliaris-Bruch's-RPE-photoreceptor complex. Each of these approaches requires the physical separation of functional neurosensory retina away from the underlying pathology, removal of CNV and haemorrhage, and restoration of the sub-retinal layers. Thus both techniques require a planned retinal detachment and reattachment.

In macular translocation, restoration of the sub-retinal anatomy is achieved by displacing the neurosensory retina relative to the underlying sclera, choroid, RPE and sub-foveal lesion. In comparison, a number of approaches to RPE transplantation have been attempted to re-establish the sub-retinal layers. These include sub-retinal injections of cell suspensions derived from autologous RPE, iris pigment epithelium and experimental modified RPE, and autologous small macular RPE rotational

patches. More recently, RPE transplantation has involved replacement of the sub-retinal defect, with a partial or full thickness choroidal-RPE graft. In the following sub-sections, each of these approaches will be reviewed further.

B Sub-Macular Surgery

With advances in vitreo-retinal surgical techniques a number of strategies developed to treat advanced neovascular AMD not amenable to medical treatment. Early treatments ranged from intra-operative lysis of CNV feeder vessels, to the pneumatic displacement or surgical removal of sub-retinal haemorrhage, and sub-macular membranectomy to remove CNV. These initial investigations developed into the sub-macular surgical trials (SST), a series of prospective randomised controlled trials designed to establish whether membranectomy or removal of haemorrhage would either stabilise or provide some improvement over the natural history of neovascular AMD. The SST Group N and Group B trials evaluated the role of surgical removal of CNV and haemorrhage in neovascular AMD (Hawkins et al., 2004b, Bressler et al., 2004).

Sub-Macular Haemorrhage

Sub-macular haemorrhage is known to have a very poor prognosis in neovascular AMD (Berrocal et al., 1996, Bennett et al., 1990). Sub-retinal haemorrhage results in an immediate central scotoma related to the size of the bleed, however the blood has a number of additional deleterious effects. These include a direct toxic effect of iron liberated from the blood on the choriocapilliaris, mechanical damage to photoreceptor outer segments from traction due to fibrin contraction, and reduced photoreceptor metabolism secondary to the blood uncoupling the photoreceptors from the RPE and acting as a diffusion barrier (Glatt and Machemer, 1982, Toth et al., 1991).

A number of techniques for the management of sub-macular haemorrhage have been evaluated without removal of concomitant CNV. Reports have described pneumatic displacement of sub-retinal blood away from the fovea using intra-vitreal gas or air and prone posturing with or without intra-vitreal recombinant tissue plasminogen activator (r-TPA) to lyse the sub-retinal clot (Hesse et al., 1999, Hassan et al., 1999, Hattenbach et al., 2001, Handwerger et al., 2001). Alternate techniques describe a pars plana vitrectomy (PPV) followed by either sub-retinal injection of r-TPA (Haupert et al., 2001, Olivier et al., 2004) or the creation of a posterior

retinotomy with direct mechanical clot extraction (with or without r-TPA) prior to gas exchange and posturing (Ibanez et al., 1995).

Most of these reports have been small case series with short follow up and poor visual outcomes despite reasonable success in anatomical clearance of haemorrhage (Table 2). While the displacement of the clot away from the fovea has obvious advantages, crucially the underlying pathology remains untreated. Furthermore the procedure requires swift intervention from onset of haemorrhage and subsequent medical intervention to prevent further disease progression – the most commonly cited cause for a failure to significantly improve vision. Additionally the use of intra-vitreal r-TPA was also questioned due to low penetrance into the sub-retinal layer and reports of retinal toxicity above 25µg/0.1ml (Johnson et al., 1990).

Sub-Macular CNV

de Juan and Machemer were the first to report sub-macular surgery for CNV and sub-retinal haemorrhage (de Juan and Machemer, 1988). They described a technique involving a PPV, creation of a posterior retinotomy, infusion of balanced salt solution and removal of the CNV. Intraocular bleeding was controlled with raised irrigation pressures or heavy perfluorocarbon liquid. A complete gas exchange with prone posturing completed the procedure. Their pilot study of 4 patients confirmed the surgical technique and resulted in improvement in vision in 3 of the patients. The technique was adopted for the SST for the treatment of CNV.

Table 2 Studies of Removal of Sub-Macular Haemorrhage

Study	Year	No. Patients	Technique	Follow Up / months	Displacement of Clot	No. Eyes with Improved Vision
Ibanez et al	1995	20	Vitrectomy + Retinotomy + Clot Extraction	10	NR	06
Ibanez et al	1995	19	Vitrectomy + Retinotomy + Clot Extraction + r-TPA	06	NR	02
Hesse et al	1999	11	Intra-vitreal Gas + r-TPA	03 – 12	09	05
Hassan et al	1999	15	Intra-vitreal Gas + r-TPA	04 – 19	15	10
Handwerger et al	2001	14	Intra-vitreal Gas + r-TPA	01 – 13	10	02
Hattenbach et al	2001	43	Intra-vitreal Gas + r-TPA	04 – 18	35	13
Haupert et al	2001	11	Vitrectomy + Sub-retinal r-TPA	01 – 15	11	09
Olivier et al	2004	29	Vitrectomy + Sub-retinal r-TPA	01 – 03	25	17

r-TPA Recombinant Tissue Plasminogen Activator
NR Data Not Reported

SST Group N Trial

The Group N trial treated patients with large sub-foveal CNV lesions, secondary to neovascular AMD, ineligible for treatment under MPS guidelines on the basis that surgical removal of the CNV would halt or delay foveal photoreceptor loss and hence result in improved vision. 454 patients were randomised to the surgical (226) or control (228) arm of the trial and followed up for at least 2 years. No statistical difference in measured visual parameters was observed between the groups at any time after 3 months of follow up (Hawkins et al., 2004b).

SST Group B Trial

The Group B trial examined CNV removal and evacuation of sub-retinal haemorrhage in patients with predominately haemorrhagic AMD lesions. The premise of the trial was that removal of blood and CNV would halt or delay macular damage from such lesions leaving a smaller affected area and hence better vision. Use of r-TPA to assist clot removal was left to the discretion of the surgeon. 336 patients were randomised to the surgical (168) or control (168) arm of the trial and followed up for 2 years. The trial found no benefit to surgery except for a small reduction in severe visual loss in patients with an initial acuity of 20/100-10/160. Surgery was not recommended as the probability of stable or improved vision was no different to that for the entire surgical group and the complication rate for surgery was considerable (Bressler et al., 2004).

The attempts in the aforementioned studies to remove CNV and haemorrhage revealed that membranectomy and removal of blood resulted in associated loss of the sub-retinal structures and without these structures visual recovery would ultimately be limited. Histopathological specimens of extracted CNV secondary to AMD frequently have a sub-RPE and RPE component (Thomas et al., 1992, Thomas et al., 1994, Grossniklaus and Gass, 1998, Grossniklaus and Green, 1998). In the SST, 84% of excised CNV demonstrated RPE involvement. Clearly without RPE the choriocapilliaris as well as the photoreceptors are lost and visual recovery is severely compromised. Thus anatomical restoration is a pre-requisite of functional recovery, given that under disease conditions a critical pool of viable photoreceptors still exists.

To recover lost function and address this problem novel surgical techniques such as macular translocation and RPE transplantation were proposed and developed.

C Macular Translocation

1 Strategies

Macular translocation has been defined as any surgery that has the primary goal of relocating the central neurosensory retina or fovea intra- or post-operatively specifically for the management of macular disease (Au Eong, 2001). Several treatment strategies have been developed to reposition the foveal photoreceptors to an area of intact choriocapilliaris-Bruch membrane-RPE. In so doing macular translocation effectively converts a sub-foveal lesion to a juxta- (1-199μm from foveal centre) or an extra- (>200μm from the foveal centre) foveal lesion that can be treated with more conventional treatments. Alternatively, macular translocation may be combined with sub-macular surgery, in which case the fovea is located to an area outside the RPE defect that commonly results from the removal of CNV.

The techniques of macular translocation were originally classified according to the size of the surgical retinotomy / retinotomies required (Au Eong, 2001). The two main approaches are:

I Macular Translocation with Large Curvilinear Retinal Incision

This technique was further sub-classified into:

- Macular Translocation with 360-degree Circumferential Retinotomy (MT360)
  or *Full Macular Translocation*

- Macular Translocation with Large but Less Than 360-degree Retinotomy

The mechanism of translocation involved the freeing of the retina from its anterior attachment via a large or 360-degree retinotomy after the creation of a segmental or complete retinal detachment. This technique allowed the

macula or entire retina respectively to pivot around the optic disc and so reposition the fovea.

II Macular Translocation with Punctate or No Retinotomy or *Limited Macular Translocation (LMT)*

This technique was further sub-classified into:

- LMT with Chorioscleral Shortening
- LMT without Chorioscleral Shortening

The mechanism of translocation involved the creation of redundancy of the retina through chorioscleral shortening following a localised retinal detachment. Chorioscleral shortening may be achieved through either chorioscleral infolding/imbrication or outfolding/outpouching. The redundant retina is subsequently displaced relative to the sclera, choroid and RPE. With infolding the retina drapes over the infolded eye wall, whereas in outfolding the internal surface of the eye wall is reduced.

An alternate method of creating retinal redundancy without chorioscleral shortening is achieved through stretching of the retina during the formation of a localised retinal detachment. Subsequent post-operative upright head posturing allows the buoyancy of an intra-vitreal gas bubble to support the superior retina while the sub-retinal fluid used to create the detachment stretches the redundant retina downwards (de Juan and Vander, 1999).

Translocation may result in foveal displacement inferiorly, superiorly, or nasally depending on the lesion characteristics (see below) and the technique employed. Temporal displacement is effectively not possible due to the attachment of the papillomacular bundle at the optic disc. The terms retinal or macular rotation applies only to procedures in which anterior retinal attachments are freed and the entire retina or macula is rotated around the optic disc i.e. translocation with large curvilinear incisions of the retina. In all other forms of macular translocation the retina remains intact and is effectively displaced rather than rotated.

Any procedure, which successfully relocates the fovea to an area outside the margins of a sub-foveal lesion, is termed ‘effective macular translocation’. The lesion size, shape and eccentricity are of less importance as the crucial determinant in achieving effective translocation is the distance between the foveal centre and the inferior or superior border of the lesion (with all points being equidistant from the temporal margin of the disc – the point of pivot) (Kubota et al., 2001). This is referred to as the ‘minimum desired translocation’. While this is conceptually the smallest foveal displacement to achieve effective translocation, in practice a larger translocation is preferred. The advantages of the fovea being sited well away from the original lesion include ensuring the underlying choriocapilliaris-Bruch membrane-RPE are healthy/disease free, lessening the chances foveal involvement in cases of recurrence, and reducing the risk of collateral damage during further treatments to the original lesion. However, large post-operative displacements are also associated with complications such as intractable diplopia/cyclotropia. In addition, there is very little evidence base for the effectiveness of extra-macular choroid and RPE in suitably maintaining long-term foveal function.

2 Techniques of Retinal Displacement

The two most common techniques of macular translocation employed are full macular translocation with 360-degree retinotomy (MT360), as based on the model first described by Machemer and Steinhorst (Machemer R, 1993a, Machemer R, 1993b) and to lesser extent, limited macular translocation (LMT), as described by De Juan and associates (de Juan et al., 1998, Imai et al., 1998). The concept of retinal relocation was first proposed by Lindsey et al (1983) (Lindsey et al., 1983) with successful shifting of the retina in rabbits and primates. Tideman et al (1985) (Tiedeman et al., 1985) later reported successful 45º rotation of the retina employing a technique involving segmental retinal detachment, a relaxing retinotomy, gas-fluid exchanges, retinal tacking, and silicone oil tamponade. This procedure was however complicated by proliferative vitreoretinopathy (PVR) and subsequent retinal detachment.

Macular Translocation with Full 360-degree Retinotomy

Almost a decade later Machemer and Steinhorst (Machemer R, 1993a) developed an animal model for translocation which was then modified for a pilot human study of three patients (Machemer R, 1993b) (Table 3). The original procedure involved the following steps: 1 vitrectomy with lensectomy, 2 creation of a complete retinal detachment, 3 360-degree retinotomy, 4 retinal rotation and reattachment. Retinal separation from the RPE was achieved by means of an infusion of lactated Ringer's solution into the sub-retinal space, accessed via a transscleral approach. A 360-degree peripheral retinotomy was then performed and the retina stabilised with a fluid-air exchange prior to retinal rotation. Rotation was achieved through repeated gentle stroking movements of the retina and the application of suction to the periphery in a tangential direction. Finally the retina was reattached with gas tamponade. The study not only demonstrated the feasibility of the technique but also confirmed through microscopic findings the restoration of the RPE-photoreceptor complex.

For the subsequent human study the technique was modified with the additional steps of removal of sub-retinal blood and membranes after retinotomy, stabilisation of the retina with perfluorocarbon prior to retinal rotation, retinal reattachment by photocoagulation of the retinotomy edge and tamponade with silicone oil. In this preliminary study PVR with retinal detachment developed in two of the three patients resulting in poor visual outcomes. A third patient improved vision from 1/200 to 20/80, however at the cost of image tilt, diplopia and cyclotropia (Machemer R, 1993b).

Table 3 Preliminary Human Translocation Studies

Study	Year	No. Patients	Technique	Follow Up / Months	Rotation Achieved	Visual Outcome	Complications %
Machemer & Steinhorst	1993	AMD 03	MT360 Lensectomy Vitrectomy Transscleral Hydrodissection Retina Total Retinal Detachment 360º Peripheral Retinotomy CNV Removed Fluid-Air Exchange Retinal Rotation Laser Photocoagulation To Retinotomy Silicone Oil Tamponade	4.8 (4.0 – 5.5)	30 – 60 º	33% > 20/100 Pre-Operative 1/200 – 2/200 Post-operative 20/80 – 3.5/200	Complications Rate: 100 33 Raised IOP 33 Hypotony 33 Cyclotropia 66 Corneal Oedema 66 PVR 66 Retinal Detachment
Ninomiya et al	1996	AMD 02 Myopia 01	MT180 Vitrectomy ± Lensectomy Segmental Retinal Detachment Temporal 180º Flap Retinotomy Removal of CNV Fluid-Air Exchange Inferonasal Retinal Rotation Laser Photocoagulation To Retinotomy Silicone Oil Tamponade	19 (10 – 28)	10 – 20 º	66% > 20/100 Pre-Operative 20/200 – 20/700 Post-operative 20/20 – 20/2000	Complication Rate: 66 100 Nasal Field Loss 33 Neovascular Glaucoma 66 PVR 33 Retinal Detachment
de Juan et al	1998	AMD 02 Angiod 01	LMT + Chorioscleral Shortening Partial Scleral Resection Vitrectomy Transretinal Hydrodissection Retina Sub-total Retinal Detachment Chorioscleral Shortening - Imbrication Fluid-Air Exchange Retinal Manipulation Gas Tamponade Post-operative Laser To CNV		350 – 1500 µm	100% > 20/100 Pre-Operative 20/126 – 20/200 Post-operative 20/30 – 20/70	Complication Rate: 66 66 Distortion 33 Cyclotropia 33 Retinal Detachment

Macular Translocation with Partial Retinotomy

To reduce the problem of poor visual outcomes associated with PVR and retinal detachment Ninomiya et al (1996) (Ninomiya Y, 1996) performed macular translocation with a smaller 180º flap retinotomy (Table 3). In this technique following vitrectomy ± lensectomy, a segmental retinal detachment was created with a sodium hyaluronate infusion. The retina was pre-treated with endodiathermy along the planned incision line that extended circumferentially from the superotemporal to the inferotemporal arcade, half way between the optic disc and equator. The pre-treated retina was cut and an 180º retinal flap elevated and reflected back to allow removal of CNV. The flap was unfolded with a fluid-air exchange and rotated with suction to the inferior edge in an inferonasal direction. This created an inferotemporal radial fold of redundant retina and a superotemporal strip of bare RPE. Following drainage of sub-retinal fluid by repeated aspiration, photocoagulation was placed along the retinotomy edge and silicone oil tamponade applied. In this pilot study of three patients, two patients achieved vision of 20/20 and 20/70 respectively, however the technique was also complicated by PVR and detachment. In addition the temporal retinotomy and area of bare RPE remaining after rotation resulted in nasal field loss in all patients and neovascular glaucoma in one case. To date three further studies performed translocation with a smaller flap retinotomy, and Table 4 summarises the visual outcomes and complications of this modification to MT360.

Limited Macular Translocation with Chorioscleral Shortening

To address the complications of macular translocation as described above (PVR, detachment, image tilt, diplopia, cyclotropia) a technique of retinal displacement avoiding large retinotomies was developed using an animal model by Imai et al (1998) employing chorioscleral shortening with infolding (Imai et al., 1998). The procedure involved the following steps: 1 partial scleral resection, 2 vitrectomy, 3 sub-total retinal detachment, 4 chorioscleral shortening, and 5 retinal reattachment. The scleral resection was crescenteric in shape, parallel to the limbus, located circumferentially in the superotemporal quadrant, and of partial (75%) thickness. A sub-total/temporal retinal detachment was created via transscleral retinal hydrodissection with an infusion of lactated Ringer's solution and chorioscleral shortening was achieved by suturing together the edges of the dissected scleral bed to create infolding. A partial fluid-air exchange was subsequently performed, and the

fovea displaced inferiorly. Ringer solution with heparin was then used to flatten the detached retina, resulting in further migration of the redundant retina away from the scleral indentation.

In the subsequent pilot human study of three patients (Table 3), de Juan et al (1998) (de Juan et al., 1998) modified the technique and the sub-total retinal detachment was created by retinal hydrodissection through small posterior retinotomies rather than a transscleral route. Furthermore, following chorioretinal shortening, a partial fluid-air exchange was performed without draining sub-retinal fluid and intraocular manipulation used to facilitate movement of the redundant retina. Finally, gas tamponade and upright post-operative posturing were used to displace the retina inferiorly and achieve retinal flattening. Following retinal reattachment the CNV was treated conventionally. In the animal study the scleral resection was placed superotemporally to produce inferior foveal displacement, however in two of the three patients, the scleral resection was placed in the inferotemporal quadrant to achieve superior foveal displacement. In this study a vision of 20/100 was achieved in all cases and there was no incidence of PVR. This method of translocation however still resulted in one patient developing a post-operative retinal detachment and distortion and cyclotropia remained problematic (Table 3).

These pilot studies confirmed the conceptual basis for foveal relocation and the feasibility of the surgical techniques. Subsequent surgery broadly proceeded along the paths of full and to a lesser extent limited macular translocation with further refinements to reduce the complication rates that limit the effectiveness of each approach. Full macular translocation offered the advantages of direct access to sub-retinal pathology and the potential for large and more controlled foveal displacements allowing foveal relocation well away from any residual disease. In contrast, limited translocation aimed to restore the same RPE-photoreceptor complex by avoiding large retinotomies and with less ocular misalignment.

Table 4 Macular Translocation with Partial Retinotomy: Visual Outcomes and Complications

Study	Year	No. Patients	Retinotomy	Improving 3 Lines VA %	VA>20/100 %	VA>20/40 %	PVR Rate %	Detachment Rate %	Additional Complications %
Ninomya et al	1996	03	180	66	66	33	66	33	33 Neovascular Glaucoma 100 Nasal Field Loss
Ohji et al	1998	05	180	40	20	20	40	20	20 Neovascular Glaucoma 60 Macular Pucker 60 Constriction Visual Field
Akduman et al	1999	20	180	00	00	00	65	65	05 Phthisis Bulbi
Ohji et al	2001	06	150	NA	17 *	17	83	83	17 Neovascular Glaucoma 67 Epi-macular Proliferation

PVR Proliferative Vitreoretinopathy
VA Visual Acuity
* Vision > 20/80

3 Evolution of Surgical Techniques

Macular Translocation with Full 360-degree Retinotomy

Several modifications of translocation surgery have taken place since the techniques of MT360 and LMT were first described. These have been driven primarily by the need to improve visual outcomes and reduce complication rates. The first major modification, as described above, was the use of a partial (180 degree / 150 degree) rather than a full (360-degree) retinotomy by Ninomyia et al (1996) (Ninomiya Y, 1996). This was intended to reduce the rate of PVR and detachment, however the four studies to date to use this modification have met with limited successes (Table 4).

Ohji et al (1998) (Ohji M, 1998) modified the technique by performing retinal detachment through an internal approach via a small retinotomy in the first case in their series. This technique was further successfully employed by Wolf et al (1999) (Wolf et al., 1999) in five of their series of seven eyes. Retinal detachment performed via an internal approach allowed a more controlled detachment and reduced the risk of retinal puncture (and the consequent risk of PVR) and failure of formation of detachment inherent in introducing the infusion cannula into the sub-retinal space from a scleral approach. This group also made more extensive use of perfluorocarbon to control the retina prior to retinal rotation and reattachment.

Eckardt et al (1999) (Eckardt C, 1999) reported the next modification to MT360 by employing advances in vitreo-retinal and performing torsional muscle surgery as either a primary or secondary procedure. Primary globe counter-rotation was performed at the beginning of translocation surgery and secondary counter-rotation at a later date depending on the strabismus findings. This was introduced to address post-operative diplopia and cyclotropia deemed to be an unavoidable side effect of translocation surgery. This study also made full use of improvements in vitreoretinal instrumentation since the first reports of MT360. This included the use of wide-angle viewing systems (BIOM, Moeller-Wedel) and scleral fixated wide-angle illumination fibre optics (TotalView Chandelier, DORC) to perform the most extensive vitrectomy possible and to minimise the trauma of surgery. Perfluorocarbons were also used as previously described but additionally to control the detached retina prior to 360º

retinotomy. Finally, the use of customised instruments, such as special atraumatic retinal forceps, further minimised surgical trauma. This technique allowed for much greater foveal shifts (Ohji et al., 2001) with vision less affected by recurrences in CNV. These modifications were reflected in the results where 18/30 (60%) patients were able to read newsprint and 22/30 (73%) patients had stable or improved vision. Furthermore all of the patients undergoing torsional muscle surgery were free of complaints of diplopia and cyclotropia.

Toth et al (2001) (Toth and Freedman, 2001) made further refinements to the technique by initially creating a single infusion micro-retinotomy and subsequently completing retinal detachment by a slow sub-retinal injection via a silicone round ball cannula held over the micro-retinotomy. In addition, this group used a two-function tissue manipulator for illumination during lesion removal and translocation, thus eliminating the need for any additional scleorstomies. Furthermore, retinal rotation was performed with a modified diamond dusted silicone tip that allowed retinal manipulation and perfluorocarbon injection for better control of the retina once placed in its new location. In this study, the fovea was always translocated superiorly unless contraindicated by the size and position of the CNV. Superior translocation was justified because: 1 PVR and detachment frequently affect the inferior retina and macula, 2 haemorrhage from larger CNV extends inferiorly to affect the RPE at an inferior site, and 3 advancement of the inferior oblique is more effective for correcting torsion following superior translocations than advancement of the superior oblique tendon for inferior translocations.

Further modifications to MT360 have included the use of calcium and magnesium free fluid instead of balanced salt solution to facilitate retinal detachment by Faude et al (1999) (Faude et al., 1999, Faude et al., 2001). This was successfully used by Aisenbrey at el 2002 (Aisenbrey et al., 2002a, Aisenbrey et al., 2002b), however reports have questioned the use of low calcium and magnesium levels on retinal (Szurman P, 2000) and RPE (Roider J, 2000) ultrastructure (Szurman P, 2000) and ERG amplitudes (Luke et al., 2001, Luke et al., 2007). Fujikado et al (2001) (Fujikado et al., 2001) also successfully used a calcium- and magnesium-free solution infused into the vitreous cavity to facilitate the creation of an intentional retinal detachment, followed by infusion of balanced salt solution into the sub-retinal space.

Pertile et al (2002) (Pertile and Claes, 2002) used specifically designed curved scissors (DORC International) to perform the 360º retinotomy. These allowed the retinotomy to be performed as peripherally as possible and avoid the variable amount of unwanted retinectomy that accompanies the use of a vitreous cutter.

Kubota et al (2003) (Kubota et al., 2003) reported a modified technique for creating total retinal detachment. After creating a small retinal detachment with a 39-gauage needle, a 20-guage silicone-tipped needle was then placed in the hole created by the needle, and balanced saline solution injected sub-retinally at a high flow rate, reducing the surgical time.

Cekic et al (2003) (Cekic et al., 2003) developed two continuous outflow devices to aid in the separation of retina from RPE and retinal manipulation. The first, a rigid needle, was developed to create a safe retinal detachment by keeping intraocular pressure low. The second, an injection needle with a soft tip facilitated injection of perfluorocarbon while manipulating the retina. Both instruments were successfully used in 54 cases.

Limited Macular Translocation

Lewis et al (1999) (Lewis et al., 1999) reported the first modification to the technique of LMT as described by de Juan et al (1998) (de Juan et al., 1998). Instead of scleral resection, imbrication was achieved by the use of mattress sutures placed in partial thickness through the sclera. The sutures were placed circumferentially between the rectus muscles close to the equator and tied after completion of the vitreous surgery. In their pilot study of 10 patients, the authors felt the technique was unpredictable in the degree of displacement achieved (114-1919μm) and compromised by complications of retinal fold formation (30%) and retinal detachment (10%). However vision improved in 40% patients and 20% achieved vision of ≥ 20/100 with post-operative image tilt and distortion persisting in only one patient.

Theoretically scleral shortening with imbrication does not create the required retinal redundancy, as the internal surface area of the globe at the level of the

RPE/choroid/sclera remains unchanged. Some of the redundant retina is draped over the ridge of infolded sclera and choroid, limiting the degree of translocation. To address this problem as well as the surgical limitations of unpredictable, minimal or no foveal displacement and the formation of retinal folds across the fovea Kamei et al (2000) (Kamei, 2000) reported the use of scleral clips to create radial outpouching to achieve chorioscleral shortening. Kamei and associates compared three different methods of scleral shortening in porcine eyes: 1 circumferential imbrication with sutures, 2 circumferential imbrication with scleral clips, and 3 radial outpouching with scleral clips. The foveal displacement achieved was 2377μm, 2582μm and 3386μm respectively with radial outpouching showing significantly more displacement. Kamei and associates also demonstrated that circumferential imbrication displayed the greatest deformation of the globe and formation of undesirable retinal folds. Similarly in a cadaveric study, Lin et al (2000) (Lin et al., 2000) used radial mattress sutures to produce significantly greater displacement with outpouching as compared with imbrication. These initial studies suggested scleral outpouching to be a more predictable and effective method of limited translocation with formation of a single retinal fold in a desired location.

Meyer et al (2000) (Meyer CH, 2000) reported the first human study that compared the degree of displacement with the differing techniques of translocation. The study reported mean distances of displacement of 1100μm for outpouching, 1710μm for imbrication, and 3718μm for MT360. Subsequently, Benner et al (2001) (Benner et al., 2001), Lewis et al (2001) (Lewis, 2001), and Kamei et al (2004) (Kamei et al., 2004) utilised the technique of outpouching for macular translocation (Table 5). Table 6 provides data for the largest consecutive series of LMT with circumferential imbrication for comparison and the results demonstrate that chorioscleral outpouching to be an effective and equivalent technique for scleral shortening.

Sullivan et al (2002) (Sullivan et al., 2002) performed scleral imbrication with a modified scleral retraction technique. In their cadaveric study they reported that use of a scleral retraction suture, which produces a double infolding of the sclera, resulted in a flatter fold compared to the standard suture technique and produced 890μm of shortening. In the three patients who subsequently underwent LMT with the modified suture, two achieved visions of 20/100 or better with three lines of improvement. The mean displacement achieved was 2003 (1400 – 2400) μm and the

technique was suggested as an adjunct to the standard suture technique, especially in patients where clips may pose a threat of perforation.

Kamei et al (2004) (Kamei et al., 2004) modified the technique of chorioscleral shortening from radial to diagonal outpouching in order to make the surgery easier and obviate the risk of a choroidal fold involving the fovea. The anatomic and visual results were again comparable to those achieved with the circumferential imbrication (Table 6) and radial/circumferential outpouching techniques (Table 5).

Counter – Rotation Surgery

An immediate complication of shifting the fovea to a new ectopic position is the creation of horizontal, vertical, and cylcotorsional strabismus. The cyclovergence amplitudes of a normal person are less than 6 degrees and it is known that individuals are unable to fuse or tolerate large degrees of cyclotorsion. Studies of patients with ocular motility disorders have shown that, in individuals with up to 15 – 20 degrees of torsion, subjective torsion disappears with monocular fixation despite persistent mechanical torsion of the eye. This is thought to be due to perceptual and proprioceptive adaptations associated with long-standing changes in the environment or by suppression if the fellow eye has good vision (Fujikado et al., 1998). The cyclotropia induced by LMT and MT180 is of the order of approximately 20 degrees, however with MT360 the degree of rotation achieved results in cyclotorsion that greatly exceeds 20 degrees (Table 3). Thus, in early studies post-operative diplopia and cyclotropia were considered to be unavoidable but tolerable side effects of the surgery.

Seaber and Machemer 1997 (Seaber JH, 1997) demonstrated the aforementioned adaptation in two patients with post-operative torsion of up to 55 degrees with use of the operated eye alone following MT360. Under binocular conditions however patients reported considerable cylcotorsional disorientation. They found the peripheral retina of the fellow eye had perceptual precedence despite poor central visual acuity, in contrast to the reduced peripheral retina of the operated eye. Hence they suggested translocation more suitable for situations in which the vision in the non-operated eye was expected to be lower than the predicted vision in the operated

eye. Furthermore patching of the fellow eye was the suggested management for cylcotorsional disorientation. This complication was subsequently addressed by simultaneous or secondary extraocular muscle surgery to counter-rotate the globe.

Counter-rotation surgery commonly involves the oblique muscles with additional rectus surgery to augment the degree of rotation. The exact nature of the muscle surgery depends upon the direction of rotation of the retina and the timing of the surgery. The fovea may be translocated superiorly or inferiorly depending on the precise location and degree of extension of the CNV. Superior translocation is the preferred method because: 1 PVR more frequently affects the inferior retina thus a superior rotation is less likely to result in retinal detachment, 2 Haemorrhage from CNV extends inferiorly potentially affecting the quality of the inferior RPE, 3 Haemorrhage from any recurrence is more likely to track across an inferiorly rather than a superiorly translocated macula, 4 A greater degree of excyclotorsion, via tucking/advancement of the inferior oblique, is possible than incyclotorsion, from advancement of the superior oblique tendon, for the correction of cyclotorsion - favouring a superior translocation.

Counter rotation surgery may be performed as a primary or a secondary procedure. The advantage of a primary procedure is that is allows for the fovea to be placed exactly such that it corresponds to the prior muscle surgery and thus avoid/minimise any post-operative cyclotropia. The disadvantages are that muscle surgery further extends the duration of surgery and does not necessarily eliminate the need for a secondary procedure due to phenomena such as unwinding of the globe. The advantage of a secondary procedure is that subsequent muscle surgery can be specifically tailored to post-operative strabismological results; the disadvantage is that patients potentially experience the disabling side effects of post-operative cyclotropia.

Eckardt et al (1999) (Eckardt C, 1999) were the first to report macular rotation with successful prevention of the disorientating cyclotropia by performing globe counter rotation both as a combined primary or a secondary procedure. As a primary procedure, the extraocular muscle surgery was carried out at the start of the procedure and involved the eye to be translocated as well as the fellow eye if

necessary. Surgery was performed on the oblique muscles to achieve a maximum of 15 degrees of cyclotorsion, with additional surgery on two or four recti to augment the degree of rotation up to 50 degrees. Depending on strabismological findings, further surgery could be performed on the operated eye or the fellow eye, including recession of the superior and lateral muscles of the fellow eye.

Eckardt and associates developed a method of torsional rectus surgery that allowed a greater degree of counter rotation than previously reported in the literature (Table 7). For superior rotation, surgery on the oblique muscles comprised a 12-mm tuck of the inferior oblique and a 12mm recession of the superior oblique. Oblique muscle surgery was augmented by surgery on the recti, which rather than conventional transposition, involved splitting the recti into 15 – 17 mm strips approximately one quarter of the muscle width. For an excyclotorsion the strips were crossed under the original muscle and moved clockwise (right eye) and counter clockwise (left eye) and repositioning them at the insertion of the adjoining rectus muscle. In their study, five patient did not undergo any extraocular muscle surgery, of which 60% reported considerable disorientation from post-operative diplopia and cyclotropia. For the twenty-five patients who underwent primary/secondary counter rotation, 96% were free of symptoms of diplopia and image tilt.

Freedman et al (2000) (Freedman et al., 2000) and Fujikado et al (2001) (Fujikado et al., 2001) later performed muscle surgery using a modification of the oblique surgery described above, where to correct for an incyclotorsion the superior oblique was recessed and the inferior oblique tucked and transposed to the upper border of the lateral rectus. Other variations to counter-rotation surgery include Freedman et al (2002, 2003) (Freedman et al., 2002, Freedman et al., 2003) who combined surgery on the oblique muscles with transposition of the lateral recti. Table 8 summarises the studies that have utilised these techniques or modifications thereof with the majority of the surgery performed as a primary combined procedure. Rates of cyclotropia greater than 15 degrees range from 0 to 23 %, with symptomatic diplopia being reported with a frequency of 0 – 33 %. These studies suggest that counter-rotation surgery is effective at reducing large degrees of cyclotorsion produced by MT360 when graded to match pre-operative torsion, however residual torsion precludes the reestablishment of binocular function and any resultant diplopia ultimately limits the overall visual success of the original surgery.

Table 5 Chorioretinal Shortening with Scleral Outpouching: Visual Outcomes and Complications

Study	Year	No. Patients	Technique of Outpouching	Displacement µm	Improving 3 Lines VA %	VA ≥20/100 %	VA ≥20/40 %	Complications %
Benner et al	2001	5	Radial Sutures	Mean 1276 (852 – 1620)	0	40	0	20 Retinal Detachment 20 Diplopia 20 Sub-retinal Haemorrhage
Lewis	2001	25	Titanium Clips Radial Sutures Circumferential Sutures	Median 1142 (0 – 3200) Median 1629 (0 – 3200) Median 614 (0 – 1326)	8	40	0	Nil Reported
Kamei et al	2004	27	Titanium Clips	Median 1576 (349 – 3391)	33	48	15	04 Retinal Detachment 15 Macular Hole 07 Retinal Tear 04 Choroidal Haemorrhage 04 Cyclotropia

VA Visual Acuity

Table 6 Chorioretinal Shortening with Scleral Imbrication: Visual Outcomes and Complications

Study	Year	No. of Patients	Technique Of Imbrication	Displacement µm	Improving 3 Lines VA %	VA≥20/100 %	VA ≥20/40 %	Complications %
Pieramici et al	2000	101	Circumferential Sutures	Median 1200 (200 – 2800)	NR	49	10	01 Choroidal Haemorrhage 09 Macular Hole 03 Macular Fold 02 Scleral Perforation 10 Retinal Tear 09 Retinal Detachment 01 Sub-retinal Haemorrhage 04 Vitreous Haemorrhage

VA Visual Acuity
NR Data Not Reported

Table 7 Methods of Torsional Rectus Surgery

Study	Year	Method of Torsional Rectus Surgery	Cyclotorsion Achieved / Degrees		
			Rectus Surgery	Oblique Surgery	Combined Surgery
Eckardt et al	1999	Transposition of Strips From Two/Four Recti To Insertion of Adjoining Recti	35	15	50
De Decker W	1990	Transposition of Vertical Recti	12	15	27
Von Noorden	1993	Transposition of Horizontal Recti	12	15	27
Spielmann A	1987	Diagonal Recession of Four Recti	15	15	30

Table 8 360-degree Macular Translocation: Management of Cyclotorsion

Study	Year	No. Patients	Method of Counter Rotation	Primary / Staged Primary / Secondary Procedure %	Final Orthoptic Status	
					Residual Cyclotropia > 15° %	Diplopia %
Eckardt et al	1999	30	Eckardt	73 / 10 / 33	17	13
Freedman et al	2000	15	Modified Oblique Surgery	40 / 27 / 00	NR	NR
Fujikado et al	2001	11	Modified Oblique Surgery	91 / 00 / 00	09	18
Ohji et al	2001	36	Eckardt	89 / 00 / 17	NR	00
Aisenbrey et al	2002	90	Modified Eckardt	59 / 24 / 22	23 *	28 *
Eckardt &Eckardt	2002	07	Eckardt	86 / 14 / 00	00	14
Fujikado et al	2002	31	Modified Eckardt	100 / 00 / 06	16	23 *
Freedman et al	2002	15	Oblique Surgery and Transposition of Vertical Recti	00 / 100 / 13	00	33

Freedman et al	2003	53	Oblique Surgery and Transposition of Vertical Recti	00 / 00 / 100	00	NR

* Prior To Secondary Surgery
NR Data Not Reported

4 Complications

The initial promise of macular translocation as an effective treatment for sub-foveal CNV has been limited by the timing and occurrence of intra- and post-operative complications. These range from mechanical intra-operative (iatrogenic retinal tears, insufficient rotation) and mechanical post-operative (retinal detachment with PVR, macular pucker, hypotony) to pathological post-operative (SRNVM disease recurrence, macular oedema) and visual post-operative (torsion, micropsia) (Table 9). Indeed the evolutions of the treatment and the various modifications to the surgical techniques outlined in the previous section have been driven by the need to minimise complications and improve visual outcomes.

Since the first report of macular translocation by Machemer and Steinhorst (Machemer R, 1993b), retinal detachment, PVR, and torsion have been identified as the major factors that limit the success of the procedure. The desire to minimise these particular complications led to the first major modifications of translocation surgery, directed primarily at reducing the size of the retinotomy – translocation with partial retinotomy (Ninomiya Y, 1996) and limited macular translocation (de Juan et al., 1998) (Table 3). These pilot studies, whilst reporting a reduced rate of retinal detachment, were markedly limited in their size (three patients) and hence the significance of their outcomes. In the absence of large scale randomised control trials, subsequent case series of translocation with partial retinotomy (Table 4) and limited macular translocation (Table 11) have failed to consistently establish significant improvements in the overall rate of complications. Of particular note, translocation with partial retinotomy offered no improvement in retinal detachment or PVR rates (Table 4). In comparison, limited macular translocation, whilst having comparable detachment and PVR rates to later modifications of full macular translocation (Table 13), is beset with a high rate of a number of complications that significantly limit the visual outcome. These include a high susceptibility to disease recurrence from insufficient translocation, diplopia, and astigmatism (Table 11).

The pivotal study to demonstrate reduced rates of post-operative retinal detachment, PVR, and torsion was conducted by Eckardt et al (1999) (Eckardt C, 1999). Eckardt and associates employed modern day vitreoretinal techniques (customised instruments, contemporary viewing and illumination systems, use of perfluorocarbons

to manipulate the retina) to perform the procedure with the most extensive vitrectomy and in a much more atraumatic manner with respect to the retina than previously described. These modifications dramatically improved PVR and detachment rates and were employed by subsequent series of full macular translocation with similar results (Table 10). This group was also the first to directly address the significant problem of post-operative torsion by performing primary or secondary globe counter-rotation surgery. In their study, this procedure eliminated symptoms of diplopia and cyclotropia and these results were replicated in subsequent studies (Table 10).

Macular translocation is complex and involved sub-retinal surgery and as described above, susceptible to a number of complications, with the accompanying risk of visual loss. The procedure has been reported to range from 3.5-6.5 hours (2000) and clearly this reflects a degree of surgical experience and improvement thereof in technique and instrumentation with time (Toth and Freedman, 2001). Consequently, this will impact upon the complication rate and this is witnessed in the improvements in complication rates reported by individual study groups over time (Eckardt C, 1999, Eckardt C, 2002) (Toth and Freedman, 2001, Toth et al., 2004, Cahill et al., 2003, Park and Toth, 2003) (Table 10). Ultimately, the effectiveness of macular translocation as a procedure is closely related to the occurrence and timing of the aforementioned complications (Tables 12-13) and minimising and managing these is as critical as achieving anatomical success during surgery.

Table 9 Reported Complications of Macular Translocation

Intra-operative	Post-operative		
	Anterior Segment	**Posterior Segment**	**Visual**
Corneal Erosion	Corneal Erosion	Ineffective Translocation	Cyclotropia / Diplopia
Retinal Adhesions	Corneal Decomposition	Macular Oedema	Aniseikonia
Insufficient Rotation	Neovascular Glaucoma	Macular Pucker	Micropsia
Peripheral Retinal Tears	Hypotony	Epiretinal Membrane	Astigmatism
Macular Hole	Cataract Dislocation IOL and Iris Capture	Chorioretinal Folds	Adaptation
Vitreous Haemorrhage	Silicone Adherence To Previously Implanted Silicone IOL	Macular Hole	
Choroidal Haemorrhage		Retinal Detachment	
Scleral Perforation		PVR	
		SRNVM Recurrence	
		Sub-foveal Haemorrhage	
		Phthisis Bulbi	
		Sub-Retinal Perfluorocarbon Droplets	

Table 10 Complication Rates of 360-degree Macular Translocation

Study	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Pucker %	Hypotony %	Glaucoma %	Cyclotropia >15° %	Other %
Machemer & Steinhorst	1993	03	66	66		33	33	33	33	
Ninomya et al*	1996	03	33	66		66		33	0	
Seaber & Machemer	1997	02	0	0					100	
Ohji et al *	1998	05	20	40		60		20		60 VF Constriction
Akduman et al*	1999	20	65	65	05	15				10 Sub-Foveal Hmge 15 Cataract 05 Phthisis
Wolf et al	1999	07	43	43		14			100	43 Cataract

Table 10 Complication Rates of 360-degree Macular Translocation

*	180-Degree Macular Translocation
VF	Visual Fields
RD	Retinal Detachment
Hmge	Haemorrhage
PVR	Proliferative Vitreoretinopathy

Table 10.1 Complication Rates of 360-degree Macular Translocation

Study	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Oedema %	Macular Pucker %	SRNVM Recurrence %	Hypotony %	Glaucoma %	Cyclotropia >15° %	Other %
Eckardt et al	1999	30	17	10		13	07	10	03		17	13 Diplopia 13 Insuff Translocation 03 Retinal Tear 03 Corneal Decomp 07 PFLC
Tano et al	2000	17	29	0	06		18					
Fujikado et al	2000	01	0	0							0	
Fujikado et al	2001	11	18	09	09						09	09 Vitreous Hmge 18 Diplopia
Toth et al	2001	26 16 † 10 ††	19 31 0	15 25 0		12	08	15				15 Retinal Tears 04 Sub-retinal Hmge 30 PFLC
Ohji et al	2001	42 36 06 *	48 42 83	19 67 11	05 06 0				02 03 0	02 0 17		11 Astigmatism 02 Bullous Keratopathy 14 Diplopia
Aisenbrey et al	2002	90	19	19	01		06	03	02		23 **	01 Corneal Decomp 06 PFLC 28 Diplopia **
Lai et al	2002	15	0	0		20	20	27				
Pertile &Claes	2002	50	0	18	02			10	02			02 Diplopia 04 Choroidal Hmge

Table 10.1 Complication Rates of 360-degree Macular Translocation

†	Evolving Technique 360-degree Macular Translocation
††	Modified Technique 360-degree Macular Translocation
*	150-Degree Macular Translocation
**	Prior To Secondary Extraocular Muscle Surgery
PFLC	Sub-retinal Perfluorocarbon

Table 10.2 Complication Rates of 360-degree Macular Translocation

Study	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Oedema %	Macular Pucker %	SRNVM Recurrence %	Hypotony %	Glaucoma %	Cyclotropia >15° %	Other %
Fujikado et al	2002	34										
Eckardt & Eckardt	2002	07	14	14			14				0	14 Diplopia
Fujikado et al	2002	31	19	10	06		07	10			16	23 Diplopia **
Park & Toth	2003	08	13	0		38		0				
Cahill et al	2003	01	0	0							100	
Abdel-Meguid et al	2003	39	26	26	05		23	08	28	08		05 Corneal Decomp 46 Diplopia
Mruthyunjaya et al	2004	61	08	08		43	23	21	03			03 Sub-foveal Hmge
Toth et al	2004	25	08	0		28	28	36				12 PFLC
Eckardt et al	2004	01	100	100								

Table 10.2 Complication Rates of 360-degree Macular Translocation

**	Prior To Secondary Extraocular Muscle Surgery
Decomp	Decompensation
Hmge	Haemorrhage
PFLC	Sub-retinal Perfluorocarbon

Table 11 Complication Rates of Limited Macular Translocation

Study	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Fold %	SRNVM Recurrence %	Vitreous Haemorrhage %	Astigmatism >5 Dioptres %	Other %
de Juan et al	1998	03	33	0				33		66 Distortion
Ohji et al	1998	05	20	0					100	40 Constriction VF
Fujikado et al	1998	02	50	0				100	100	50 Diplopia 50 Cataract
Lewis et al	1999	10	10	0		30				10 Retinal Break 10 Distortion
Fuji et al	2000	124	17	12	08	05		03		08 Retinal Break 01 Choroidal Hmge 02 Scleral Perforation 02 CNV Retinotomy Site
Pieramici et al	2000	101	09	0	09	03		04		38 Insuff Translocation 10 Retinal Break 01 Choroidal Hmge 02 Scleral Perforation 02 CNV Retinotomy Site 01 Sub-retinal Hmge
Fujikado et al	2000	01	0	0			100			

Table 11 Complication Rates of Limited Macular Translocation

VF	Visual Fields
Hmge	Haemorrhage

Table 11.1 Complication Rates of Limited Macular Translocation

Study	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Fold %	SRNVM Recurrence %	Hypotony/ Glaucoma %	Astigmatism >5 Dioptres %	Other %
Glacet-Bernard et al	2001	32	22	0	17	9	43			26 Diplopia
Ohji et al	2001	9	0	11	0			0	78	22 Insuff Translocation
Benner et al *	2001	5	20	0						20 Diplopia 20 Sub-retinal Hmge
Fujii et al	2001	22	26	0	13	09	09			30 Insuff Translocation 13 Retinal Break 09 Sub-retinal Hmge
Fujii et al	2001	1	0	0						
Roth et al	2001	1	0	0						
Lewis *	2001	25	0	0			0			32 Insuff Translocation

Table 11.1 Complication Rates of Limited Macular Translocation

Hmge Haemorrhage

* Chorioscleral Shortening with Outpouching

Table 11.2 Complication Rates of Limited Macular Translocation

Author/Year	Year	No. Patients	RD %	PVR %	Macular Hole %	Macular Fold %	SRNVM Recurrence %	Astigmatism >5 Dioptres %	Other %
Sullivan et al	2002	03	0	0					66 Cataract
Hamelin et al	2002	14	14	0	07		14		21 Insuff Translocation 14 Diplopia
Fujii et al	2003	04	0	0					
Chang et al	2004	08	0	0			0		13 Cataract 13 Diplopia 13 CNV Retinotomy Site 13 Retinal Tear
Albini et al	2004	01	0	0					13 Diplopia
Au Eong et al	2004	02	0	0	50			0	50 Diplopia
Pawlak et al	2004	29	17	14	14	14	45		08 Insuff Translocation 41 Cataract 14 Diplopia
Kamei et al *	2004	27	04	0	15			0	07 Retinal Tear 04 Choroidal Hmge
Ng et al	2004	29	19	0	16	06			23 Insuff Translocation 06 Retinal Break 10 Sub-retinal Hmge

Table 11.2 Complication Rates of Limited Macular Translocation

* Chorioscleral Shortening with Outpouching

Table 12 360-degree Macular Translocation: Visual Outcomes and Retinal Detachment + Proliferative Vitreoretinopathy Rates

Study	Year	No. Patients	RD %	PVR %	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Ohji et al *	1998	05	20	40	40	20	20
Akduman et al *	1999	20	65	65	0	0	0
Wolf et al	1999	07	43	43	14	14	0
Eckhardt et al	1999	30	17	10	13	67	23
Tano et al	2000	17	29	0	35 **	NR	NR
Fujikado et al	2001	11	18	09	45	73	36
Ohji et al	2001	36 06 *	42 83	67 11	16	29 *** 17 ***	08 17
Aisenbrey et al	2002	90	19	19	27	NR	NR
Lai et al	2002	15	0	0	13	73	27

Table 12 360-degree Macular Translocation: Visual Outcomes and Retinal Detachment + Proliferative Vitreoretinopathy Rate

RD	Retinal Detachment
PVR	Proliferative Vitreoretinopathy
VA	Visual Acuity
NR	Data Not Reported
*	MT 180
**	Gain 0.3 logMAR
***	Vision > 20/80

Table 12.1 360-degree Macular Translocation: Visual Outcomes and Retinal Detachment + Proliferative Vitreoretinopathy Rates

Study	Year	No. Patients	RD %	PVR %	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Pertile & Claes	2002	50	0	18	NR	58	18
Eckardt & Eckardt	2002	07	14	14	14	86	14
Fujikado et al	2002	31	19	10	NR	52	29
Park & Toth	2003	08	13	0	NR	NR	NR
Abdel-Meguid et al	2003	39	26	26	33	15	03
Mruthyunjaya et al	2004	61	08	08	30	52 †	08
Toth et al	2004	25	08	0	NR	52 †	NR

VA Visual Acuity
NR Data Not Reported
† Vision ≥ 20/80

Table 13 Limited Macular Translocation: Visual Outcomes and Retinal Detachment + Proliferative Vitreoretinopathy Rates

Study	Year	No. Patients	RD %	PVR %	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Ohji et al	1998	05	0	11	40	40	20
Lewis et al	1999	10	10	0	10	20	0
Fuji et al	2000	124	17	12	NR	52	NR
Pieramici et al	2000	101	09	0	NR	49	10
Glacet-Bernard et al	2001	32	22 22	0 0	17 22	17 44	04 11
Ohji et al	2001	09	0	11	NR	22 **	0
Benner et al *	2001	05	20	0	0	40	0
Fujii et al	2001	22	26	0	23	68	09
Lewis *	2001	25	0	0	08	40	0

Table 13 Limited Macular Translocation: Outcomes and Retinal Detachment + Proliferative Vitreoretinopathy Rates

VA	Visual Acuity
NR	Data Not Reported
*	Chorioscleral Shortening with Outpouching
**	VA > 20/125

Table 13.1 Limited Macular Translocation: Outcomes and Retinal Detachment and Proliferative Vitreoretinopathy Rates

Study	Year	No. Patients	RD %	PVR %	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Fujii et al	2002	70	NR	NR	NR	68	NR
Hamelin et al	2002	14	14	0	50	71 **	21
Chang et al	2003	08	0	0	13	75	75
Pawlak et al	2004	29	17	14	38	19 **	04
Kamei et al *	2004	27	04	0	33	48	15
Ng et al	2004	29	19	0	27 †	54	08

Table 13.1 Limited Macular Translocation: Outcomes and Retinal Detachment and Proliferative Vitreoretinopathy Rates

RD	Retinal Detachment
PVR	Proliferative Vitreoretinopathy
VA	Visual Acuity
NR	Data Not Reported
*	Chorioscleral Shortening with Outpouching
**	VA > 20/125
†	>4 Lines Improvement

5 Effectiveness

The effectiveness of any treatment may be judged by a wide variety of criteria however common central goals of any intervention are to halt and ideally reverse disease progression, to restore a physiological state in terms of anatomy and function, and to achieve a positive impact with respect to the quality of life of the patient. Translocation surgery aims to attain these goals by directly treating the sub-foveal pathology (CNV, haemorrhage, fluid) and restoring retinal anatomy. The ideal endpoint with respect to the treatment of macular disease is therefore to provide stable central vision of good quality. The effectiveness of full and limited translocation to date is considered below along these lines, by examining the impact upon several aspects of retinal function as well as visual outcomes.

Retinal Function and Surgery

Macular translocation, particularly with 360-degree retinotomy and retinal rotation, involves extensive surgery, during which the retina is placed under a considerable degree of stress. Several factors have been identified which influence the anatomical and functional outcome of translocation surgery. These include the number of retinal photoreceptors cut during retinotomy, the amount of retina treated with photocoagulation, the speed and duration of retinal detachment, intra-operative mechanical, light and ischaemic stress, and the type of irrigating solution applied during surgery. Several studies have investigated clinicopathological correlates examining the effects of surgery on various aspects of retinal function; the key findings are detailed below.

It has been established that the extent, duration, and height of retinal detachment correlates with visual outcome and electroretinogramd (ERG) responses after reattachment (Kim et al., 1996). Luke et al (2001) (Luke et al., 2001) examined electrophysiological changes before and after MT360 and noted a significant reduction in photopic a- and b-waves after surgery. This study also observed a positive correlation between the duration of surgery and the amplitude reduction in ERG responses. Terasaki et al (2002) (Terasaki et al., 2002) also reported similar functional alterations in full-field ERGs after MT360. These studies suggest impairment of photoreceptor outer segments following MT360 and this is supported

by a number of ultrastructural studies demonstrating a loss of photoreceptor outer segments after translocation (Machemer R, 1993a, Lafaut et al., 2000). These results therefore suggest that to optomize retinal function with MT360, the emphasis is on minimising the duration of detachment and the overall surgical time.

Later work by Terasaki et al (2004) (Terasaki et al., 2004) examined focal macular, rather than full field, ERGs following MT360. This group demonstrated improvements in focal macular ERGs that correlated well with visual outcome indicating that macular function was recoverable. Thus, while MT360 may cause a significant global electrophysiological decrease, this study suggested that macular function was less affected, preserved or restored.

Another factor that influences the functional result after translocation surgery is the retinal trauma incurred during the detachment process. This is partly influenced by the type or composition of irrigation used to create the retinal detachment. The use of calcium- and magnesium-free fluid instead of balanced salt solution has been shown to facilitate and accelerate detachment (Faude et al., 2001, Szurman P, 2000, Faude et al., 1999). Szurman et al (2000) (Szurman P, 2000) injected a calcium-free buffered solution into the sub-retinal space in rabbits and demonstrated reduced retinal adhesion. However, this study also found swelling and fragmentation of photoreceptor outer segments with partial loss of the plasma membrane. It is proposed that outer segments are torn away or damaged during the detachment process. To further reduce this trauma several modifications have been introduced to the detachment stage of the surgical technique including the use of silicone ball-tipped cannula (Toth and Freedman, 2001) and continuous outflow devices (Cekic et al., 2003) for improved sub-retinal infusion. Thus, techniques to minimise the degree of neuroretinal trauma would seem appropriate, however continued research is required to determine their importance with respect to morphological and functional recovery following MT360.

Cekic et al (2004) investigated the effects of translocation surgery on the retinal circulation. This study demonstrated that choroidal perfusion parameters in the new foveal region did not change after MT360 (Cekic et al., 2004b). Further work by this

group also quantified retinal circulation times before and after surgery and found that MT360 did not alter the retinal macrocirculation in the long term (Cekic et al., 2004a).

The effect of surgery on the morphology of the relocated macula was examined by Terasaki et al, 2003 (Terasaki et al., 2003). Pre and post-operative optical coherence topography revealed the newly located macula after MT360 had a normal concave foveal configuration with normal thickness. Thus, the retinal appears to adequately adapt to the extensive mechanical insult of translocation surgery.

Visual Outcomes and Surgery

The distance visual outcomes for full thickness and limited macular translocation surgery are listed in Tables 14 and 15 respectively. As previously discussed the effectiveness of translocation is closely related to the timing and occurrence of complications and this is reflected in the visual outcomes.

When considering only series with 10 patients or more treated for neovascular AMD, full macular translocation shows improved mean visual outcomes compared to limited translocation for percentage improvement in 3 lines of visual acuity (23.9 vs. 18.3 %) and the percentage with a visual acuity greater than 20/100 (46.3 vs. 37.4 %) (Table 16). When the percentage with a visual acuity greater than 20/40 is examined, full translocation shows a significantly improved outcome over limited translocation (15.4 vs. 3.6 %) (Table 16).

Traditionally, distance acuities have dominated as an outcome measure for investigations of the eye, however near vision and reading ability are one of the most important visual tasks. Reading ability relies on a number of factors including, contrast sensitivity, stable fixation, size, density and position of a central scotoma, and position of the preferred retinal locus in relation to any scotoma (Legge et al., 1992, Sunness et al., 1996, Crossland et al., 2004a, Crossland et al., 2005a). In terms of near acuity and reading speeds, very few studies have used these parameters to document visual outcomes for macular translocation surgery, and these are outlined in Table 17 (Eckardt et al, 1999; Fujikado et al, 2002; Abdel-

Meguid et al, 2003; Mruthyunjaya et al, 2004; Toth et al, 2004). Finally, Cahil et al (2005) demonstrated MT360 was associated with improvements in vision-related quality of life measures (National Eye Institute Visual Function Questionnaire). The degree of improvement was greatest in patients with a post-operative improvement in vision and not related to a change in the general health of the subjects (Cahill et al., 2005).

The results outlined in Tables 14-16 strongly suggest that despite being more involved surgery, full translocation offers direct treatment of sub-foveal disease, together with physiological restoration of retinal anatomy and function. The studies discussed above indicate that the retina, considering the upheaval of the neurosensory layer in full translocation, is minimally impaired (in terms of vascular status, morphology, electrophysiology), and when successful results in positive functional recovery. In contrast, limited translocation, whilst being relatively less disruptive surgery does not treat sub-foveal disease, is equally vulnerable to sight threatening complications such PVR, retinal detachment and diplopia and is much more susceptible to disease recurrence. This thesis will go on to examine in detail full macular translocation, the feasibility of the technique, the anatomical restoration of foveal anatomy, and the functional success in terms of quantitative and qualitative recovery of vision.

Table 14 360-degree Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Machemer & Steinhorst	1993	03	AMD 03	4.8 (04– 5.5)	1/200 – 2/200	20/80 – 3.5/2000	33	33	0
Ninomya et al *	1996	03	AMD 02 Myopia 01	19.0 (10 – 28)	20/200 – 20/700	20/20 – 20/2000	66	66	33
Seaber & Machemer (Seaber JH, 1997)	1997	02	AMD 02	27.5 (24 – 31)	20/200 – CF	20/80 – 20/200	50	50	0
Ohji et al *	1998	05	AMD 04 Myopia 01	25.6 (04 – 48)	20/70 – 20/700	20/20 – 20/1000	40	20	20
Akduman et al *	1999	20	AMD 20	8.0 (02 – 12)	20/80 – 20/800	20/200 – PL	NR	0	0
Wolf et al	1999	07	AMD 07	11.0 (06 – 16)	20/80 – HM	20/100 – 20/400	14	14	0

VA Visual Acuity
AMD Age Related Macular Degeneration
* 180-Degree Macular Translocation
** Gain 0.3 logMAR
NR Data Not Reported
CF / HM / PL Counting Fingers / Hand Movements / Perception of Light

Table 14.1 360-degree Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Eckardt et al	1999	30	AMD 30	10.6 (03 – 18)	20/40 – HM	20/30- HM	13	67	23
Tano et al	2000	17	AMD 17	6.7 (03 – 13)	NR	NR	35 **	NR	NR
Fujikado et al	2000	01	Myopia 01	10	20/150	20/30	100	100	100
Lüke et al	2001	32	AMD 32	12	20/40 – 20/200	Mean logMAR decrease 0.3 lines over baseline	NR	NR	NR
Fujikado et al	2001	11	Myopia 11	6.2 (03 – 13)	20/50 – 20/670	20/22 – 20/330	45	73	36

VA Visual Acuity
AMD Age Related Macular Degeneration
** Gain 0.3 logMAR
NR Data Not Reported
CF / PL / HM Counting Fingers / Perception of Light / Hand Movements

Table 14.2 360-degree Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	PreOp Vision	PostOp Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Toth et al	2001	26	AMD 26 16 † 10 ††	12 (06 – 24) 14 10	Mean 20/125 20/100	Mean 20/250 20/80	NR	NR	NR
Ohji et al	2001	42 36 06*	AMD 42	06	≤20/40	>20/40 - <20/400	NR 16 NA	26 29 *** 17 ***	10 08 17
Aisenbrey et al	2002	90	AMD 90	12	Median 20/200 20/50 – HM	Median 20/200 20/40 – HM	27	NR	NR
Lai et al	2002	15	AMD 15	12	Mean 20/90 Median 20/70 20/40 – 20/800	Mean 20/64 Median 20/70 20/20 – 20/200	13	73	27
Pertile & Claes	2002	50	AMD 50	21 (12 – 36)	20/60 - <20/200	>20/50 - <20/200	NR	58	18
Fujikado et al	2002	34	AMD 23 Myopia 11	7.6 (04 – 16)	20/40 - <20/400	20/20 – 20/300	NR	68	21
Eckardt & Eckardt	2002	07	Dry AMD 01	9.7 (03 – 18)	20/60 – 20/100	20/25 – 20/200	14	86	14

Table 14.2 360-degree Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
AMD	Age Related Macular Degeneration
GA	Geographic Atrophy
NR	Data Not Reported
HM	Hand Movements
*	150-Degree Macular Translocation
***	Vision > 20/80
†	Evolving Technique 360-degree Macular Translocation
††	Modified Technique 360-degree Macular Translocation

Table 14.3 360-degree Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %
Fujikado et al	2002	31	AMD 21 Myopia 09	10 (07 – 17)	20/40 – 20/600	20/20 - <20/400	NR	52	29
Park & Toth	2003	08	AMD 08 †††	10 (04 – 23)	Mean 20/160	Mean 20/125	NR	NR	NR
Cahill et al	2003	01	GA 01	06	20/100	20/32	100	100	100
Abdel-Meguid et al	2003	39	AMD 39	12	Mean 20/260 20/60 - PL	Mean 20/260 20/40 – HM	33	15	03
Mruthyunjaya et al	2004	61	AMD 61	12	Median 20/125	Median 20/80	30	52 ***	08
Toth et al	2004	25	AMD 25	12	Mean 20/125	Mean 20/100	NR	52 ***	NR
Eckardt et al	2004	01	AFVD 01	22	20/100	20/100	0	100	0

Table 14.3 360-degree Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
AMD	Age Related Macular Degeneration
GA	Geographic Atrophy
AFVD	Adult Familial Vitelliform Dystrophy
NR	Data Not Reported
HM / PL	Hand Movements / Perception of Light
***	Vision > 20/80
†††	**Post Photodynamic Therapy**

Table 15 Limited Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥20/100 %	VA ≥ 20/40 %	Foveal Displacement /µm
de Juan et al	1998	03	AMD 02 Angiod 01	06 (04 – 08)	20/126 - 20/200	20/30 – 20/70	100	100	33	350 – 1500
Ohji et al	1998	05	AMD 03 Myopic 02	6.6 (04 – 10)	20/50 – 20/200	20/30 – 20/500	40	40	20	0.15 – 0,7 DD
Fujikado et al	1998	02	Myopia 02	06	20/70 – 20/150	20/20 – 20/30	100	100	100	0.46 – 0.63 DD
Lewis et al	1999	10	AMD 10	06	Median 20/111 20/50 – 20/800	Median 20/160 20/80 – 20/640	10	20	0	Median 1286 114 – 1919
Fuji et al	2000	124	AMD 101 Other 23	06 10.8 (06 – 18)	NR	NR	NR	49 52	NR	NR
Pieramici et al	2000	101	AMD 102	06	NR	NR	NR	49	10	Median 1200 200 – 2800

Table 15 Limited Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
AMD	Age Related Macular Degeneration
DD	Disc Diameters
NR	Data Not Reported

Table 15.1 Limited Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %	Foveal Displacement /µm
Fujikado et al	2000	01	Myopia 01	12	20/70	20/40	100	100	100	0.5 DD
Glacet-Bernard et al	2001	32	AMD 23 Myopia 09	09 (06 – 14) 10 (06 – 15)	20/40-<20/200 20/100-<20/200	20/30 - <20/200 20/100 - <20/200	17 22	17 44	04 11	Mean 1105 Mean 685
Ohji et al	2001	9	AMD 09	06	≤20/40	20/40- <20/400	NR	22 *	0	Mean 1120 250 – 2700
Benner et al	2001	5	AMD 04 GA 01	14.4 (13 – 16)	Mean 20/180 20/80 – 4/200	Mean 20/140 20/60 – 6/200	0	40	0	Mean 1276 852 – 1620
Fuji et al	2001	22	Myopia 11 POHS 04 Angiod 04 Idiopath03 MC 01	10.8 (06 – 18)	Mean 20/150	Mean 20/100	36 25 0 33 0	64 75 50 67 100	9 0 0 0 100	Mean 854 Mean 1925 Mean 1088 Mean 816 1200

Table 15.1 Limited Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
DD	Disc Diameters
NR	Data Not Reported
AMD	Age Related Macular Degeneration
GA	Geographic Atrophy
POHS	Presumed Ocular Histoplasmosis Syndrome
Angiod	Choroidal Neovascularisation Associated with Angiod Streaks
Idiopath	Idiopathic Choroidal Neovascularisation
MC	Multifocal Choroiditis
*	Vision > 20/125

Table 15.2 Limited Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %	Foveal Displacement /µm
Fujii et al	2001	1	POHS 01 (Post SMS)	04	20/100	20/40	100	100	100	NR
Roth et al	2001	1	Angiod 01	06	20/125	20/40	100	100	100	844
Lewis †	2001	25	AMD 25	06	Median 20/188 20/80 – 20/400	Median 20/173 20/64 – 20/400	08	40	0	Median 1142 0 – 3200
Fujii et al	2002	70	AMD 71	5.5	Mean 20/180 20/30 – 2/200	Mean 20/100	NR	68	NR	Mean 1523 500 – 3500
Sullivan et al	2002	03	AMD 03	06 (02 – 12)	20/200	20/60 – 20/400	66	66	0	Mean 2033 1400 – 2400
Hamelin et al	2002	14	Myopia 14	11 (06 – 24)	<20/50 - <20/200	Mean 20/100	50	71 *	21	Mean 695 100 – 1520

Table 15.2 Limited Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
POHS	Presumed Ocular Histoplasmosis Syndrome
SMS	Sub-Macular Surgery
Angiod	Choroidal Neovascularisation Associated with Angiod Streaks
AMD	Age Related Macular Degeneration
NR	Data Not Reported
†	Chorioscleral Shortening with Outpouching
*	Vision > 20/125

Table 15.3 Limited Macular Translocation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre-Op Vision	Post-Op Vision	Improving 3 lines VA %	VA ≥ 20/100 %	VA ≥ 20/40 %	Foveal Displacement /µm
Fujii et al	2003	04	AMD 04 (Post PDT)	6.75 (06 – 08)	Mean 20/190 20/150– 20/200	Mean 20/100 20/40 – 20/150	25	50	25	Mean 1525 1400 - 1600
Chang et al	2003	08	AMD 08	12	20/30- <20/150	20/30- <20/200	13	75	75	Mean 1.1 mm 0.8 – 1.5 mm
Albini et al	2004	01	AMD 01	24	20/60	20/25	100	100	100	2mm
Au Eong et al	2004	02	Myopia 01 Idiopath 01	12	20/50 – 20/100	20/30 – 20/40	50	100	100	NR
Pawlak et al	2004	29	AMD 29	14	20/70 – 20/200	NR	38	19 *	04	Mean 1274 250 – 1900
Kamei et al ††	2004	27	AMD 17 Myopia 07 Idiopath 03	27	Mean 20/174 20/50 – CF	Mean 20/167 20/25 – HM	33	48	15	Median 1576 349 – 3391
Ng et al	2004	29	AMD 24 Myopia 02 POHS 03 PXE 02	06	20-40 – CF	20/32 – HM	27 **	54	08	Median 1100 0 – 2230

Table 15.3 Limited Macular Translocation: Distance Acuity Outcomes

VA	Visual Acuity
AMD	Age Related Macular Degeneration
Idiopath	Idiopathic Choroidal Neovascularisation
POHS	Presumed Ocular Histoplasmosis Syndrome
PXE	Pseudoexanthicum Elastica
NR	Data Not Reported
*	Vision > 20/125
**	>4 Lines Improvement
††	Diagonal Outpouching

Table 16 360-degree and Limited Macular Translocation: Comparison of Distance Acuity Outcomes

Method of Translocation	Visual Outcomes / Mean Percentage *		
	Improving 3 Lines VA	VA ≥ 20/100	VA ≥ 20/100
Full	23.9	46.3	15.4
Limited	18.3	37.4	3.6

* Mean Outcomes From Series Of 10 Patients Or More (Tables 14 and 15) Treated For Age Related Macular Degeneration

VA Visual Acuity

Table 17 360-degree Macular Translocation: Near Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Mean Pre-Op Near Acuity	Mean Post-Op Near Acuity	Mean Pre-Op Reading Speed wpm	Mean Post-Op Reading Speed wpm	Distance Acuity ≥ 20/40 %
Eckardt et al	1999	30	AMD 30	10.6 (03 – 18)	21% (0.4 or better)	60% (0.4 or better)			23
Fujikado et al	2002	31	AMD 21 Myopia 09	10 (07 – 17)	0.80 0.87	0.82 0.48	115	140	29
Lai et al	2002	15	AMD 15	12	20/90	20/64	69	87	27
Mruthyunjaya et al	2004	61	AMD 61	12	0.70*	0.44*	71	105	08
Toth et al	2004	25	AMD 25	12	3.20†	1.50†	41	67	NR

* Median Near Acuity
† Meter (M) Scale

D RPE Transplantation

1 Strategies

The RPE maintains retinal homeostasis by occupying a critical and strategic position as a physical, metabolic, and regulatory intermediary between the neurosensory and underlying vascular structures of the retina. In this capacity, healthy RPE is crucial to facilitating the function of photoreceptors and hence providing good vision. This intimate relationship has led to the concept of replacement of the RPE as a therapeutic option, particularly with respect to recent advances in our understanding of the involvement of the RPE in modulating the surrounding extracellular and cellular environment, and in the pathogenesis of retinal disease at a bio-molecular level.

As discussed, the choriocapilliaris-Bruch's-RPE axis is subject to cumulative damage in susceptible individuals, leading to the development of the dry and wet forms of AMD. Historically, treatments for wet AMD have moved away from vaso-destructive towards pharmacological interventions directed primarily at arresting a single component of the final pathogenic pathway of the disease process, namely CNV formation. In view of this, cell based therapies, designed to restore the choriocapilliaris-Bruch's-RPE-photoreceptor axis, have emerged and offer the possibility of not only a physical restoration of structure and recovery of lost function, but of a potential cure in terms of improving the condition of already diseased cells and so offering long term stable vision. In this respect, we can begin to consider the concept of healthy RPE replacement as a treatment modality.

RPE transplantation reinstates the choriocapilliaris-Bruch's-RPE-photoreceptor axis that is crucial to the vision. Over the past two decades a number of strategies have been employed, initially with animal models and more recently with human studies. Effective transplantation requires, an appropriate and viable cell source, a safe and repeatable method of delivery, the ability to surmount issues surrounding potential rejection, and recovery of structure and function. To this end a number of approaches have been undertaken including the use of pigment epithelium from

sources other than the retina, homologous and autologous grafting, the use of RPE cell suspensions and full thickness sheets of RPE, and pedicled macular flap RPE grafts as well as free peripheral RPE patch grafts.

Of the approaches mentioned above, autologous full thickness free patch grafts, harvested from the mid-periphery of the retina, have become the technique of choice to treat both primary RPE and photoreceptor driven retinal dystrophies as well as more complex diseases such as AMD. Briefly, the technique involves the creation of a localised foveal detachment through a small retinotomy, removal of any CNV, followed by replacement of the RPE ('partial thickness transplantation') or the choriocapilliaris-Bruch's-RPE complex ('full thickness' transplantation) with autologous tissue. Both approaches re-align viable photoreceptors with healthy supporting tissue improving photoreceptor function and provide the opportunity to treat any CNV directly.

The following sections, will review the progress that has been made to date in this field, including background animal studies and the evolution and effectiveness of the techniques. RPE transplantation offers a vast opportunity for a new generation of more directed retinal treatments, and the scope for a curative 'halo' effect upon the neurosensory retina.

2 Animal and Cell Based Human Studies

Animal studies have served to explore a number of key areas for achieving successful transplantation. These include determining the ideal source of cell for investigation and transplantation, optimising methods for the collection and delivery of RPE, understanding the mechanisms involved in the assimilation transplanted material, establishing the degree of physiological restoration of the RPE layer in terms of anatomy and function, and examining the extent and stability of any recovery or reversal of RPE driven retinal disease.

Sources of Cell for Transplantation

A number of sources of RPE have been used for transplantation (Table 18). Early studies in mammalian species, initially employed adult (Gouras et al., 1984), and later embryonic RPE cells (Del Priore et al., 2003b) in autologous, homologous and heterologous approaches. With improvements in cell culture and genetic engineering, modified RPE cells became available with the ability to study the effect of growth factors, and mark and follow the effects of RPE and retinal cellular metabolism (Abe et al., 2005, Abe et al., 1999). More recently, iris pigment epithelium, which is embryonically derived from the same neuroepithelium as the RPE, has been transplanted to the retina, and recent advances in stem cell technology have exploited progenitor retinal and neural stems to provide RPE for transplantation (Warfvinge et al., 2006, Haruta et al., 2004) .

Techniques of Cell Delivery

Transplantation first became possible with improvements in RPE cell culture and the concomitant detailed understanding of RPE ultrastructure, morphology, biochemistry and physiology (Flood et al., 1980, Flood et al., 1984, Boulton et al., 1982). As a result of this work, cultured RPE cells were transplanted into the sub-retinal space of simian eyes, initially using an open-sky technique (Gouras et al., 1984, Gouras et al., 1985), Whilst allowing straightforward access to the sub-retinal space, the open-sky approach was limited by the inability to reattach the neurosensory retina. Thus, whilst RPE attachment to Bruch's membrane could be studied, the effect of the donor RPE cells in a restored physiological state could not. To circumvent this difficulty,

investigators adopted closed techniques of RPE cell delivery that offered a much less disruptive end result. Two methods of closed RPE cell delivery were described; predominantly in animal studies, an external approach was used to access the sub-retinal space and inject RPE cells through a posterior trans-scleral route (Li and Turner, 1988b). In contrast, the internal approach delivered RPE cell suspensions to the sub-retinal space via a trans-retinal route. This involved a pars plana vitrectomy, creation of a small retinotomy, and the injection of RPE cell suspensions through a micropipette into the sub-retinal space (Lopez et al., 1987, Brittis et al., 1987) or the introduction of larger RPE patch grafts with explant injectors (Thumann et al., 2006) or with aspiration-reflux spatulas (Maaijwee et al., 2007). The closed internal approach provided a number of advantages by offering a more controlled method of cell delivery that did not result in any trauma or inflammation to the choroid or Bruch's membrane, and mirrored techniques of vitreoretinal surgery in humans.

Techniques of Cell Collection

A number of techniques have evolved for the collection of RPE cells for either culture or transplantation. Initial reports gathered RPE cell suspensions by enzymatic degradation from enucleated eyes with the cells isolated by centrifuge (Flood et al., 1984, Flood et al., 1980). The first report to describe a method for the harvesting and storing of intact viable sheets of RPE cells was described by Tenzel (1997). Intact sheets of RPE were enzymatically cleaved from their basement membrane and embedded in 50% gelatin. The study demonstrated that sheets of RPE cells were suitable for retinal transplantation if harvested within 24 hours of death and maintained 82% viability for as long as 48 hours if stored at 4 degrees C (Tezel et al., 1997).

Table 18 Sources of Retinal Pigment Epithelium for Animal Transplantation Studies

Study	Year	Source Cell	Graft Classification	Technique
Gouras	1984	Adult RPE	Heterologous	Open
Li and Turner	1988	Adult RPE	Homologous	Closed – External
Lopez et al	1987	Adult RPE	Homologous	Closed – Internal
Del Priore	2003	Embryonic RPE	Heterologous	Closed – Internal
Abe	2005	Transformed RPE	Homologous	Closed – Internal
Schraermeyer	2000	Iris Pigment Epithelium	Homologous	Closed – Internal
Warfvinge	2006	Retinal Progenitor Cells	Heterologous	Closed – Internal
Haruta	2006	Embryonic Stem Cell	Heterologous	Closed – Internal

The aforementioned work referred to homologous RPE transplantation, whereas for autologous transplantation, the RPE cells are required to be harvested from the same eye. Early studies used an external trans-scleral method to gather RPE cells (Lane et al., 1989, Wongpichedchai et al., 1992), however more recently, trans-retinal approaches have been adopted, for the benefits outlined above, to collect either RPE cell suspensions (Phillips et al., 2003) or full and partial thickness RPE patch grafts (Maaijwee and van Meurs, 2006).

Graft Integration

A number of studies have identified several factors that lead to the successful assimilation and subsequent function of transplanted RPE into host tissue. These include the presence of a substrate on which to receive the cells, namely a healthy Bruch's membrane or a synthetic equivalent, suitable extracellular matrix or scaffold, and the expression of appropriate cell adhesion molecules.

Early studies established that epithelial cells fail to survive in suspension and that separating RPE from its extracellular matrix induces apoptosis. This apoptosis was prevented when RPE was harvested onto extracellular matrix, fibronectin or laminin (Tezel and Del Priore, 1997), suggesting that prior to transplantation these cells must rapidly reattach to a substrate. The natural substrate for RPE cells is Bruch's membrane and it has been demonstrated that the ability of transplanted RPE cells to repopulate Bruch's membrane depends on the age of the donor tissue and the ultrastructural layer available for reattachment, both reflecting the state of any pre-existing disease.

As previously mentioned a number of ageing changes are known to occur at the choriocapilliaris-Bruch's-RPE interface related to the deposition and accumulation of lipid-protein aggregates. These deposits affect the ability of the choroidal blood supply to adequately support the photoreceptors. In fact early studies have demonstrated that metabolic impedance or conductance across Bruch's membrane naturally declines with age (Starita et al., 1995). This is thought to be secondary to tissue remodeling, with the process being accelerated by lipid deposition in AMD (Starita et al., 1996), and localised to the inner collagenous layer (Starita et al.,

1997). These studies suggested that any potential substrate for RPE required adequate conductance, or treatment of the pre-existing substrate, the inner collagenous layer of Bruch's, to improve conductance to sustain photoreceptor function.

The apoptosis rate of attached cells has been shown to increase with attachment to deeper layers of Bruch's membrane (Tezel et al., 1999, Tezel and Del Priore, 1999a). Later studies with RPE cell repopulation of human Bruch's membrane attributed similar results to age-related changes in the inner collagen layer. Subsequent treatment of Bruch's to determine the effect of cleaning and/or an extracellular matrix protein coating (a mixture of laminin, fibronectin and vitronectin) on the reattachment, apoptosis, proliferation, and final surface coverage of the transplanted RPE cells revealed that the age-related changes in the inner collagenous layer that impair repopulation could be reversed (Tezel et al., 2004). Similarly treatment of the donor tissue has been shown in studies to modify RPE attachment. Studies with porcine RPE, where the TGF-beta family of cytokines mediates the density-dependent growth suppression of RPE in vitro, demonstrated that neutralizing these cytokines by adding anti-TGF-beta antibodies could result in more rapid growth of the RPE in vivo (Tezel and Del Priore, 1999b). From this work it is clear that such biological modifications of the graft and the recipient site offer future targets for successful transplantation therapies

A body of in vitro and in vivo work has also examined various artificial substrates for RPE cells. These have ranged from biological membranes such as porcine lens capsule (Turowski et al., 2004), human amniotic membranes (Ohno-Matsui et al., 2006), and sheets of cross-linked collagen (Bhatt et al., 1994) or fibrinogen (Oganesian et al., 1999), to synthetic materials such as ployetherurethanes (Williams et al., 2005). Although all these studies have demonstrated improved ability to support RPE in terms of outcome measures such as cellular morphology, phenotypic differentiation, restoration of a monolayer, rejection/inflammation and photoreceptor survival over naïve RPE cells, as yet no single substrate has demonstrated the flexibility, robustness, biostability and biocompatibility to allow secure delivery and transplantation.

To circumvent difficulties with rejection and make use of Bruch's as a natural substrate, autologous RPE transplantation utilizes a full thickness mid-peripheral RPE-choroidal graft with its own intact substrate (van Meurs and Van Den Biesen, 2003). These grafts were originally placed on debrided sub-foveal Bruch's in a porcine model and the graft successfully revascularised via bridging vessels from the underlying choroids to the choriocapilliaris of the graft. The grafts survived for 12 weeks and it is postulated that the adjacent proximity to the choroidal blood supply is sufficient to maintain RPE survival whilst revascularisation is established between the donor and recipient sites (Maaijwee and van Meurs, 2006).

In addition to an intact Bruch's membrane, cell adhesion molecules have also been shown to be vital to successful RPE transplantation. In particular, the cadherins and integrins are essential in RPE cell adhesion to Bruch's membrane and surrounding cells to form a uniform monolayer. Cadherins are adhesive molecules found at adherens and desmosomes and are implicated in cell-cell adhesion and cellular orientation. Integrins are transmembrane glycoproteins that posses subunits which are receptors for collagen, laminin, and fibronectin – all of which are components of Bruch's membrane (Hynes, 2002). The integrins have also been implicated in determining cell polarity and retinal adhesion (Nandrot et al., 2008), phagocytosis of photoreceptor outer segments (Feng et al., 2003, Finnemann et al., 1997), and interacting with the complement system to aid adhesion (McLaughlin et al., 2003).

Graft Success

The aim of transplantation is to rescue failing macular function and in vivo animal models of transplantation have utilized the Royal College of Surgeons (RCS) rat to examine photoreceptor function. The RCS rat carries a RPE mutation resulting in the loss of photoreceptor outer segments phagocytosis. The subsequent accumulation of sub-retinal debris leads to a rod-cone dystrophy and RPE transplantation has been shown to reverse these effects (Lund et al., 2001b, Li and Turner, 1988a, Coffey et al., 2002, Wang et al., 2005a). Using this model early RPE transplantation studies demonstrated RPE cells established a normal relationship with photoreceptors and that there was histological rescue of the neuroretina (Li and Turner, 1988b, Li and Turner, 1988a). In addition to the photoreceptors, RPE transplantation was also shown to maintain the underlying vasculature and the choriocapilliaris in particular

(Majji and de Juan, 2000). Later RCS studies with RPE allo- and xeno-grafts further demonstrated functional recovery using physiological and behavioral testing (Lund et al., 2001a, Coffey et al., 2002). RPE transplantation was able to recover complex visual function such as centre-periphery distinction, identifying directional change, contrast sensitivity and pattern discrimination (Coffey et al., 2002, McGill et al., 2004).

From animal studies the obvious mechanism for the documented recovery is direct contact between RPE cells and the immediate layers above and below. Early studies with cell suspensions and later with sheets of autologous transplantation demonstrated that the formation of a monolayer is a prerequisite for successful transplantation. As discussed above this relies on correct cellular orientation, adequate association between the RPE and its substrate, and correct binding between neighbouring RPE cells and the surrounding intercellular matrix. Beyond this a number of trophic factors have also been implicated in successful transplantation. These include basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF). Trophic factors explain findings where recovery extends beyond the immediate graft area, where recovery occurs when RPE cells have been introduced into the vitreous cavity, and where recovery occurs when transplantation involves glial tissue (Castillo et al., 1997, Sauve et al., 2002, Wang et al., 2005b, Abe et al., 2005)

The degree to which RPE transplantation in animal studies has sustained the outcomes described above has varied depending upon a number of factors. When considering the tissue transplanted from an immunological viewpoint, rejection will clearly affect graft survival. Although the sub-retinal space is considered a privileged site, the anatomical disruption secondary macular disease, particularly AMD, and from the tissue delivery process itself, compromises this situation. In this respect, autologous transplants are more advantageous than allo- and xeno-grafts, however the latter two methods allow more scope tissues to be modified prior to transplantation. For example, as mentioned above, the gene encoding BDNF has been transduced into RPE delivered to the sub-retinal space in a rat model with successful neuroprotection of the retina (Abe et al., 2005). This model allows for RPE cells to act as a vehicle for gene replacement therapy.

A number of animal studies have investigated the role of the host immune system in allogenic and xenogenic grafts. In rats, RPE allografts have survived from 2 to 12 months with chronic rejection occurring and an incompatibility in MHC class I and II antigens identified as a limiting factor (Zhang and Bok, 1998, Li and Turner, 1991). Similarly, in rat studies using xenografts from human and porcine RPE sources, grafts have survived from 2 to 10 months prior to rejection (Grisanti et al., 2002, Abe et al., 1999, Coffey et al., 2002, Wang et al., 2005b). In these studies the sub-retinal RPE xenografts are protected from a strong cellular rejection, with an absence of lymphocytes, but seem to undergo a slow functional deterioration, reflected by a decline in their capability to rescue adjacent photoreceptors. Despite the absence of an acute lymphocytic response, allograft and xenograft studies have identified an increase in the production of interleukins (IL-1 and IL-6) (Abe et al., 1999, Enzmann et al., 2000), as well as a glial and macrophage response (Del Priore et al., 2003a, Lai et al., 1999, Gabrielian et al., 1999). This inflammatory response appears be modifiable by systemic and local immunosuppression, (Del Priore et al., 2003a, Lai et al., 2000, Wang et al., 2002), as well as the use of a RPE substrates (Oganesian et al., 1999, Bhatt et al., 1994), although in no such study has a graft survived beyond 12 months. Thus, in allogenic and xenogenic graft transplantation the immune response remains one of the most important limiting factors.

Other factors identified as determinants of graft survival include the number of cells transplanted, the age of the donor cells, and time between RPE cells being harvested and transplanted (Castillo et al., 1997, Li and Turner, 1991). The success of the graft is also dependant upon the degree of residual photoreceptor function in the host and the secondary downstream structural losses resulting from the disease process.

Human Transplantation Studies

The large body of animal studies demonstrating successful transplantation of RPE onto Bruch's membrane, with associated photoreceptor survival and or functional recovery, together with a lack of treatment modalities for human retinal diseases, led a number of investigators to examine the feasibility of human RPE transplantation. A number of retinal abnormalities are potentially treatable by RPE transplantation, including neovascular AMD, geographic atrophy and some macular dystrophies.

Historically, poor outcomes for patients with CNV led to the development of early sub-macular surgical techniques directed at sub-retinal membranectomy and removal of haemorrhage (de Juan and Machemer, 1988, Blinder et al., 1991, Thomas et al., 1992, Thomas et al., 1994). Histological examination of the excised CNV revealed concomitant loss of RPE and the dissected sub-retinal bed was incompletely repopulated with proliferating or migrating RPE (Grossniklaus et al., 1994, Lopez et al., 1991, Hsu et al., 1995). The loss of RPE support was secondarily associated with choriocapilliaris atrophy and loss of photoreceptors (Korte et al., 1984, Pollack et al., 1996, Akduman et al., 1997). The results of these early studies were later confirmed by the sub-macular surgical trials (Hawkins et al., 2004a, Bressler et al., 2004, Hawkins et al., 2004b, Grossniklaus and Green, 1998). Together with animal studies, this body of work further supported the need for RPE replacement. Subsequently human transplantation studies have predominantly focused on treating neovascular AMD with the rationale that RPE repopulation of the dissected bed restores sub-retinal anatomy and provides the necessary metabolic and trophic support to the neuroretina.

Techniques of Transplantation

The first report of human RPE transplantation was presented by Peyman et al 1991 for end stage AMD (Peyman et al., 1991). In their study they described two techniques, one case involving homologous transplantation with adult RPE and a second involving an autologous macular RPE-choroid rotational flap. Using vitreoretinal techniques previously outlined, the study described pre-operative 360-degree photocoagulation at the ora and surrounding the arcades, the preparation of a large retinal flap created within the margins of the pre-operative laser, removal of the sub-macular scar, and replacement of the RPE cells, using either a full thickness autologous pedicle graft, or a homologous adult RPE patch with Bruch's membrane and choriocapilliaris. Following retinal reattachment, 360-degree endolaser was applied with silicone oil tamponade and an encircling band. Fourteen months following the procedure, visual acuity in the patient with a pedicle graft had improved from counts fingers to 20/400 and the patient fixated over the transplanted RPE cells. After 10 months, the homologous graft in a second patient had become encapsulated with a fine sub-retinal membrane without neovascular tissue and visual acuity had not improved. This finding was thought to be secondary to presumed graft rejection. No intra-operative or post-operative complications resulting from the surgery were reported in either patient.

From this first reported human case, methods of RPE harvesting and surgical techniques of cell delivery have developed along the lines of the type of transplantation undertaken, namely homologous or autologous. Over the last decade the later has prevailed, driven primarily by the practicalities of obtaining suitable tissue for transplantation, and the advantages of avoiding issues of tissue matching and rejection inherently present with homologous transplantation.

Homologous Foetal Transplantation

The aforementioned case of homologous transplantation described by Peyman et al (1991) utilized a sheet of RPE from a patient who had suffered a traumatic eye injury requiring enucleation. Subsequent studies of transplantation for AMD have harvested RPE, as sheets or cell aggregates, from both adult and foetal sources. Gouras et al (1994) successfully demonstrated that foetal RPE cells could be cultured by removing small patches of this layer from the choroid of the foetal eye. These RPE patches were cultured to give rise to healthy, epithelioid monolayers in vitro within 1-2 weeks, with correct polarity and the ability to phagocytose outer segments. Furthermore the patches were capable of being lifted off a confluent monolayer and transferred to another culture dish without risking the viability of either the old or the new culture, and so providing a means of transplanting an organized, polarized patch of human RPE from one place to another (Gouras et al., 1994).

A cultured foetal RPE patch (15-17 weeks gestational age) was subsequently employed by Algerve et al (1994)) (Algvere et al., 1994). In their study, five patients with AMD (pre-operative visual acuity 0.08-0.2) underwent removal of sub-retinal fibrovascular membranes using pars plana vitrectomy techniques. The foetal RPE was cultured (2-7 days) and transplanted as a rolled monolayer patch into the sub-retinal space via a cannula through the retinotomy employed for CNV removal. Following reattachment, endotamponade was achieved with either gas or silicone oil. Three RPE transplants were placed in the fovea and two were placed in a para-foveal location. All transplants survived for 3 months and were found to increase in size to cover part of the epithelial defect caused by removal of the fibrovascular membrane. SLO microperimetry indicated that visual function was present in four of the transplants at 1 month but in only two at 3 months after surgery. Function over the transplants, especially those in the fovea, was compromised by cystoid-like macular oedema. The latter complication was thought to be the result of chronic rejection and a compromised blood/retinal barrier.

A later study by the same group (Algerve et al, 1997) investigated whether human RPE allografts were tolerated or rejected in the sub-retinal space and also examined the feasibility of RPE transplantation in subjects with exudative and non-exudative AMD (Algvere et al., 1997). This study employed both patches (delivered as a rolled

sheet via a cannula), as well as cell aggregates (delivered as cell suspensions via a micropipette) of foetal cultured RPE (13-20 weeks of gestational age), transplanted via a small retinotomy into the sub-retinal space of five patients after surgical removal of sub-foveal fibrovascular membranes, and to four subjects with dry geographic atrophy. Suspensions of RPE cells were transplanted to four other patients with non-exudative AMD. In disciform lesions, RPE transplants developed macular oedema concomitant with gradual reduction of visual acuity, implying host-graft rejection, over 1-6 months. In geographic atrophy, three of four transplants showed little change in shape and size after 12 months with one transplant slowly rejected. In non-exudative AMD, RPE suspension transplants showed no evidence of rejection and were associated with the disappearance of drusen; visual acuity remained stable and SLO microperimetry confirmed retinal function over the transplanted area. They concluded that human RPE allografts were not invariably rejected in the sub-retinal space without immunosuppression, with lower rejection occurring in non-exudative than exudative AMD. Once again an intact blood-retinal barrier was thought to protect against rejection and it was felt that it was technically feasible to transplant human RPE into the sub-macular space without adversely affecting visual function in non-exudative AMD over relatively long periods of time.

Similarly Weisz et al (1999) reported a case of foetal RPE transplantation for end stage geographic atrophy (Weisz et al., 1999). In their report a suspension of RPE cells was infused via a micropipette into the sub-retinal space through a retinotomy along the superotemporal arcade at the edge of the area of atrophy. No endotamponade was applied. The patient's vision remained unchanged for 5 months after the surgery. Although the feasibility of foetal RPE transplantation to the sub-retinal space was again confirmed subsequent investigations revealed leakage and staining at the level of the outer retina, a progressive sub-retinal fibrosis in the area of the transplant, and a concerning lymphocyte response against phosducin and rhodopsin.

Homologous Adult Transplantation

Following on from the original case report by Peyman et al (1991), and on the background of a number of animal studies demonstrating RPE cell viability on artificial substrates, Tezel et al (1997) described a method for the harvesting and storing of intact viable sheets of adult human RPE cells (Tezel et al., 1997). The cells were harvested from 21 cadavers eyes using the enzyme Dispase. The sheets were embedded in 50% gelatin containing 300 mM sucrose and stored at 4 degrees C. The viability of the cells, as well as their ability to proliferate in vitro, was studied for 96 hours after harvesting. Subsequent microscopic examination of the RPE sheets confirmed the cells appeared suitable for retinal transplantation if harvested within 24 hours of death and maintained 82% viability for as long as 48 hours if stored at 4 degrees C. The study confirmed the feasibility of isolating and harvesting intact sheets of viable adult human RPE using the enzyme Dispase.

This technique was subsequently utilized by Del Priore et al (2001) in a case report of adult human transplantation (Del Priore et al., 2001). An 85-year-old patient with bilateral choroidal neovascularisation underwent sub-foveal membranectomy combined with transplantation of a sheet of human adult RPE under the foveal centre in the right eye. The RPE sheet was delivered and unfolded via a cannula through the retinotomy utilized for CNV removal and following retinal reattachment an air tamponade was applied. The patient was immunosuppressed post-operatively with prednisone, cyclosporine, and azathioprine. The patient died from congestive heart failure 114 days after surgery with a lack of visual improvement. Subsequent histological examination revealed the transplant site contained clusters of round, pigmented cells that did not form a uniform monolayer in most areas. There was loss of the photoreceptor outer segments and native retinal pigment epithelium in the centre of the transplant bed, with disruption of the outer nuclear layer predominantly over regions of multilayered pigmented cells. Cystic spaces were also present in the inner and outer retina. The morphological findings were consistent with the clinical outcome.

Progressing on from successful enzymatic harvesting of RPE cells, Valtink et al (1999) proposed RPE cell banking, akin to a cornea bank, as a prerequisite to have well-differentiated, characterized cells to hand when required for research or

therapeutic purposes. This group enzymatically isolated RPE cells from donor eyes and cultured them for subsequent HLA typing, cryopreservation in liquid nitrogen, and registration on a donor list (RPE cell bank) (Valtink et al., 1999b). 116 typed cell cultures were stored as a source for 7 subsequent cell transplantations with matching HLA type in patients suffering from RPE degenerative diseases (Valtink et al., 1999a).

The homologous transplantation studies detailed above have shown limited success in terms of restoration of function, with foetal RPE transplantation for non-exudative disease providing the most positive outcomes. Likely explanations for the poor clinical outcomes include the significant difficulties associated with tissue matching and rejection, but additionally, the RPE cell loss secondary to the need for cell harvesting and preservation prior to transplantation, the surgical learning curve, and the method of RPE delivery with increased overall cellular manipulation over autologous techniques. Considering the latter, the above studies have delivered homologous RPE to sub-retinal locations, through small retinotomies used initially to create localized detachments. The RPE is introduced either as sheets or patches via a cannula and unfolded, or as cell suspensions via a micropipette. The advantage of using sheets of RPE is that a pre-formed correctly orientated monolayer can be specifically placed over the diseased area. This is however offset against the technical difficulties encountered when delivering a relatively large sheet through a small retinotomy. The sheet will require additional manipulation to mount it (usually rolled) on or into the cannula or micropipette and then correctly unfold and position it to prevent multi-layering and folding, although foetal and gelatin fixed patches unfold spontaneously. This can be circumvented by creating a large flap retinotomy as per Peyman et al (1991) however this warrants the need to create a much more extensive detachment adding additional complexity and time to the procedure. An alternate method of RPE delivery is to inject cell suspensions into the sub-retinal space. While this is a far simpler method, it has the disadvantage that the formation of a monolayer cannot be guaranteed, with cells incorrectly orientated or not attaching to Bruch's membrane and entering the vitreous cavity. As mentioned, despite the poor functional outcomes, the body of work on homologous transplantation provided considerable information on techniques of cell harvesting, culture and viability, for future studies examining artificial RPE substrates and stem cell derived sources of RPE.

Autologous Transplantation

Autologous transplantation has a number of advantages over homologous transplantation. Primarily it avoids the problems of tissue compatibility and rejection by harvesting RPE cells from the patient and relocating to the diseased area, there is less overall manipulation of the graft and associated cell loss, there is no risk of transmitting any disease from one patient to another, and it avoids ethical and religious conflicts arising from using foetal or cadaveric sources of RPE.

In the first description of autologous human transplantation Peyman et al (1991) fashioned a large retinotomy and rotated a full thickness pedicle graft into position. Stanga et al (2001) employed a similar technique, however surgery was performed through a small retinotomy and all but one of the study patients underwent transplantation with a free rather than a pedicle graft (Stanga et al., 2001). The small retinotomy offered the advantage of reducing nerve fibre and subsequent filed loss from a large retinotomy, reduced risk of PVR, no need for pre-operative laser, and reduced surgical time. In their study, six patients with untreated exudative AMD underwent surgical excision of the sub-foveal CNV with RPE translocation and were followed from 1 to 10.5 months. The surgery consisted of a standard vitrectomy with excision of the CNV through a small retinotomy and a localized detachment. Using vertical sub-retinal scissors introduced through the retinotomy a 500-1500μm pedicled full thickness RPE-choroid graft was fashioned from the edge of the RPE defect in the first patient and a free graft in the remaining five patients. Once fashioned, the grafts were immediately positioned under the fovea to maintain the correct RPE orientation, again using the vertical scissors, with gas tamponade following reattachment. The study demonstrated that RPE could be effectively translocated, at the time of CNV removal, from the edge of the RPE defect to a sub-foveal location. Optical coherence tomography (OCT) showed the translocated RPE as an area of increased optical reflectivity with optical shadowing external to it and graft autofluorescence revealed viable translocated RPE. Microperimetry was performed with a cross fixation target and this was seen when projected on the translocated RPE. Photopic 10-2 perimetry, photopic fine matrix mapping, and confocal laser scanning ophthalmoscopy (cLSO) confirmed the presence of central visual function. At an average follow up of 4.5 months, the visual acuity remained stable in all but one patient (83.3%), with no patients improving their vision. The

complications reported in the study included two patients in whom it was not possible to place the patch under the desired retinal location, one with an inverted graft, one patient with a sub-retinal bleed, and one patient developing PVR with a total retinal detachment. The study confirmed that para-foveal transplanted RPE was capable of supporting limited photoreceptor function in patients with AMD and that the absence of a pedicle suggested revascularization of the graft was a possibility, although there was limited follow up on patients receiving the free graft.

A second study by Stanga et al (2002) further investigated the feasibility of this technique and the visual function over the translocated RPE with longer follow up (12 to 32 months) (Stanga et al., 2002). In addition to the six previously treated, three further patients with untreated exudative AMD underwent surgical RPE translocation as detailed above however the technique was slightly modified. In the second study, the sub-retinal scissors created two sides of a triangular graft and a second retinotomy was created to cut the third side. Once again a pedicle graft was fashioned for the first patient with free grafts for the remainder. Long term follow up of this cohort revealed surgery was successful in seven of nine patients. The recorded complications remained the two patients with inadequate graft placement, the one patient with an inverted graft, and the one patient who experienced a total PVR with detachment. Post-operative investigations confirmed viable transplanted tissue with the presence of central visual function. At an average follow up of 19.7 months follow up, visual acuity remained stable in 6 patients (66.7%), deteriorated in two patients (22.2%) (one uncomplicated surgery and the PVR detachment), and improved in one patient (11.1%) (CF to 6/60). Significantly, the improvement occurred with a free graft with 12 months follow up, providing strong indirect evidence for graft revascularization. The results of both studies demonstrated that not only was autologous transplantation technically possible, but in addition to anatomical restoration, it also offered real functional recovery.

A long term follow up of the cohort of patient who underwent successful transplantation from the above study was performed by McLaren et al (2005) (MacLaren et al., 2005). Four of the 9 patients who originally underwent surgery and whose results were reported after 2 years of follow-up were reviewed again 5 to 6 years after surgery. Visual acuity, imaging, angiography, and the maintenance of overlying foveal fixation were used to assess the long-term success with

comparisons made to the original 2-year follow-up data. The assessment revealed that over the long term, vision had declined further in 3 patients and improved slightly in 1 patient. Significantly, all 4 patients had lost foveal fixation and autofluorescence of the graft, which had been present at the original 2-year follow-up assessment. The RPE graft, however, seemed viable when assessed by OCT and angiography, and no patient suffered a recurrence of the CNV. The study confirmed anatomical graft survival in the sub-foveal space for at least 5 to 6 years, although functional rescue appeared to be transient. It was postulated that the long-term loss of graft autofluorescence, foveal fixation, and ultimately vision might be secondary to chronic photoreceptor apoptosis, initiated either by the original trauma of surgery, or a continuation of the disease process itself. Conversely the graft or trophic factors released by it may have slowed the natural course of disease driven apoptosis, and the loss of autofluorescence simply reflect the death of overlying photoreceptors secondary to loss of disc shedding. It was also suggested that as the graft was harvested from an extra-foveal region, it was likely to be diseased and compromised from the outset. Thus, the placement of diseased RPE under the densely packed foveal photoreceptors may have overburdened the graft leading to apoptosis. Finally, the use of extra-foveal RPE also created a defect in an area that would otherwise have been used as an alternate fixation point when foveal fixation is lost. In this regard visual loss was compounded after graft failure and this was in fact found to be the case, as the one patient who improved vision had reestablished fixation at the edge of the central defect. This study suggested that while macular RPE may survive and initially support photoreceptor function, ultimately this method of transplantation was unable to sustain long-term recovery of function.

Using a similar technique to that outlined by Stanga et al (2001), Angunawela et al reported a 4 year follow up on a pedicled graft (Angunawela et al., 2005). The graft was fashioned in the sub-retinal space with vertical scissors and repositioned successfully under the fovea following CNV removal. The case was complicated with a PVR detachment however angiography revealed the graft remained vascularised at 4 years and the patient maintained counting fingers vision. This study also confirmed long-term anatomical graft survival however no detailed functional analysis of the graft was offered. The RPE rotation techniques described above, and early macular translocation studies (Machemer R, 1993b, Eckardt C, 1999) provided the strongest proof of principle that non-foveal, autologous RPE from eyes with AMD could to a limited degree support foveal function.

The next stage in the evolution of autologous RPE transplantation was the use of peripheral rather than macular RPE. This was first presented by Binder et al (2002) in the form of sub-macular injections of nasally harvested RPE cell suspensions (Binder et al., 2002). The study was based on previous observations of RPE loss associated with neovascular AMD, the fact that the nasal retina contains more RPE than the other quadrants and is much less involved in this disease process, the fact that Bruch's membrane may be locally fragmented rather than absent, and on the requirement for RPE replacement to maintain photoreceptor viability. The early homologous studies showed no visual improvement and late immune reactions necessitating immunosuppression, and the autotransplantation studies detailed above showed sequestration after long periods of observation, although both Peyman et al (1991) and Stanga et al (2001, 2002) demonstrated some recovery of function. These rotation techniques had a relatively high complication rate and this prompted Binder et al (2002) to look for a technical solution that could provide fresh autologous RPE with reduced risk. In addition to density of RPE, the nasal retina was chosen for ease of controlled RPE cell aspiration and mobilization to a sub-foveal site, and a secure area for a retinotomy with respect to subsequent tamponade. In a prospective study, 14 eyes (13 patients) with neovascular AMD (2 classic; 2 occult; 10 mixed lesions; all had angiographic growth of lesion between 3 and 9 months previously with substantial acuity decline and baseline acuities ranged between CF and 20/50 {6 out 14 had VA < 20/200}) underwent sub-retinal CNV removal with simultaneous transplantation of RPE harvested from the nasal sub-retinal area of the same eye. Following standard sub-retinal techniques for CNV excision, a second retinotomy was created nasal to the optic disc with a sub-retinal pick and a shallow detachment created. With a blunt bent 20 gauge vitreoretinal cannula, RPE cells were gently mobilized over a 2-4 disc diameter area and the cell suspension aspirated with a micropipette connected to a tuberculin syringe. Two thirds were transplanted slowly through the first retinotomy created for CNV removal and to avoid reflux of cells at the time of transplantation the intraocular pressure was further lowered. One third of the RPE cells were sent for cell differentiation and if the amount of cells was low (<5000/ml), the procedure was repeated. Finally, the vitreous was cleared of any visible pigment cells and the surgery completed with gas tamponade and prone posturing for a few days. Post-operatively, at the 12 month follow up point, best-corrected visual acuity was improved 2 or more lines in eight eyes (57.1%), remained the same (+/- 1 line) in five eyes (35%), and decreased by more than 2 lines in one eye (7.1%). Pair wise t test showed significant improvement after 1 month (P = 0.0031, P = 0.0062) as well as 1 year (P = .0066, P = .0105). Satisfactory

reading vision between Jaeger 1 and 4 was achieved in three eyes (21.2%) and no significant intra-operative or post-operative complications were reported including recurrence of CNV. The study however was not able to confirm the optimal number of cells for transplantation, whether the functional results observed were a direct effect of the transplanted cells restoring the photoreceptor-RPE-choriocapilliaris axis or an indirect effect whereby transplanted cells were supporting existing RPE function as bystanders, either directly or through the secretion of trophic factors, and whether adequate cellular confluency was required or achieved. The study added to the preceding body of work suggesting that autologous RPE transplantation with sub-macular surgery was a reasonable treatment option for neovascular AMD

Binder et al (2004) reported a second consecutive series of 39 eyes (predominantly occult lesions) in which they slightly modified their surgical technique to further improve cell delivery and minimise any potential complications (Binder et al., 2004). In the later study once mobilized, the RPE cells were removed from the eye, centrifuged and diluted with BSS plus solution, and then injected under the retina with the additional use of perfluorocarbon to reduce RPE cell reflux during sub-retinal delivery. Cell counts were performed as per the original study to assess cell numbers and quality, and finally, if required, laser was applied to the retinotomy. The second study design compared membrane excision and transplantation (group 1 – 39 eyes) with membrane excision alone (group 2 – 14 eyes) and in addition to distance acuity outcomes, also examined functional recovery through multifocal electroretinography (mfERG) and reading ability. In group 1, visual acuity improved significantly, two or more lines in 21 (53.8%) patients; remained stable in 12 patients (30.8%); and decreased two or more lines in 6 patients (15.4%; P=0.0062). In group 2, the corresponding values were 21.1%, 57.8%, and 21.1% (P=0.5377). Overall, the net change was gain in 1 line in the control group compared with 2 lines in the transplanted group. In addition, the difference in reading acuity was also significant between the two groups (mean change in group 1: 1.85 +/- 0.42 vs. 0.43 +/- 0.47 in group 2; P=0.0001). Furthermore mfERG response density changes were significantly different between groups 1 and 2 (P=0.0094) although no significant decreases in central visual field defects were detected. The reported complications included 4 cases of PVR detachment (10.2%) presumed to be due to reopening of the retinotomy with reflux of RPE cells. The study further confirmed that CNV removal with autologous RPE transplantation of RPE was a beneficial supplement to

membrane excision alone in patients with neovascular AMD and may be regarded as a reasonable treatment option.

A modification of the above technique for harvesting RPE cell suspensions was proposed by van Meurs et al (2004) (van Meurs et al., 2004) in a series of eight consecutive patients treated for neovascular AMD. In this method, three weeks pre-operatively, laser photocoagulation was used to delineate an area of inferior peripheral retina for RPE cell harvesting. Three hours prior to surgery, blood was collected from the patient to obtain autologous serum. Subsequent surgery involved, lensectomy if phakic, vitrectomy, and CNV excision. An inferior retinectomy was performed to minimize the effects of any resulting scotoma and RPE cells were collected from this location using a modified bent 21 gauge aspiration cannula with an 8-O nylon loop to scrape RPE off Bruch's membrane and simultaneously aspirate the cells. The pre-operative laser retinopexy partly overcame any disadvantage from a lack of subsequent inferior tamponade. The RPE cells were then removed from the eye, centrifuged with autologous serum to prevent cell adhesion to the tubing, and poly-L-lysine injected into the sub-macular space to promote adhesion of the RPE cells to Bruch's membrane. The cells were counted and tested for viability and then injected into the sub-retinal space and perfluorocarbon used to control RPE cell reflux. The surgery was completed with air, gas, or silicone oil tamponade and one day of supine posturing followed by prone posturing. The results revealed that at a follow up ranging from 3 months to 16 months, the vision remained stable in five patients (62.5%) and deteriorated in three (37.5%). A pigmented area was observed in the transplantation site in only one patient and fixation was present at the edge of the RPE defect in three patients, but not over the pigmented area in the only patient with visible pigmentation. PVR detachment was the major complication occurring in the three patients with a reduction in vision and this was thought to be secondary to the creation the large retinectomy with aspiration of RPE cells leading to breakdown of the blood-retinal barrier, unsuccessful prevention of RPE reflux with perfluorocarbon, reflux of poly-L-lysine promoting RPE adhesion to the retina, and the inferior retinectomy coming into contact with the aqueous phase (containing inflammatory cells) when using lighter than water tamponades. With this technique autologous peripheral RPE cell transplantation after membrane extraction was technically possible in a sterile manner, but was associated with a high proliferative vitreoretinopathy rate. Furthermore, RPE cell repopulation was only observed in one patient and the study revealed no measurable positive effect on functional outcome.

This led to the author abandoning this technique and proposing transplantation using full thickness patches harvested from the mid periphery.

The most current method of autologous RPE transplantation was first described by van Meurs and Biesen (2003) (van Meurs and Van Den Biesen, 2003). With the limited results achieved using diseased macular RPE patch grafts, and the difficulties of using suspensions that do not adequately attach to aged or damaged Bruch's or fail to form a satisfactory monolayer, it was postulated that transplantation of an intact peripheral monolayer would be required to achieve a functional transplant. Van Meurs and Biesen proposed a technique that involved harvesting a full thickness RPE-choroid patch from the superior mid periphery and placing it via a retinotomy under the fovea following CNV excision. Briefly, following vitrectomy, the CNV was excised through a small para-macular retinotomy and the donor site for the patch graft (1.5 – 2.0 mm) delineated with endodiathermy, which also avoided any potential choroidal haemorrhage. The retina was subsequently removed from within the donor site and, using vertical scissors, a patch of RPE-choroid was fashioned from within the diathermy marks. Prior to graft delivery the host site was prepared by injection of BSS plus into the sub-macular space to maintain the height of the localized detachment originally created to remove the CNV. Using a spatula, the free patch was then repositioned under the fovea through the para-macular retinotomy. This delivery technique was used only for the first three patients as it was found that the elasticity of the RPE-choroid caused the graft to roll up into a cigar shape requiring considerable manipulation to unfold and correctly place it. Thus, for the next two patients, graft capture and delivery was achieved with customised forceps, and thereafter with a specially designed aspiration-reflux cannula (DORC Surgical Instruments, The Netherlands). The latter, requiring a bimanual technique, involved the graft being aspirated onto the end of a modified flat spatula and introduced by the surgeon through the retinotomy to the desired sub-retinal position. The graft was then released from the spatula by gentle reflux and simultaneous injection of perfluorocarbon by the assistant to flatten the localized macular detachment and maintain the sub-retinal position of the graft. The advantages of this technique being that it minimized the trauma to the graft, it ensured correct orientation of the graft, and it allowed a more controlled release of the graft. The disadvantages being attempted graft repositioning is cumbersome if the graft unfolds incorrectly, requiring reversal of the aforementioned steps, and added graft manipulation and so graft positioning is essentially a one shot procedure. Other disadvantages include graft

damage secondary to delivery through a small retinotomy, similarly the retinotomy limits the size of the graft resulting in inadequate coverage of the RPE defect and a loss of revascularization if this in part relies on contact with healthy RPE-choroid at the edge of the defect, and the graft becoming entangled around the cannula preventing smooth release. The surgery was completed with laser photocoagulation to the mid-peripheral retinotomy site and silicone oil tamponade. The oil was removed at three months combined with cataract extraction and lens implantation.

In their study, van Meurs and Biesen treated six patients with neovascular AMD (minimally classic) with this technique. The pre-operative visual acuity ranged from 20/400 to 20/200 with an average duration of visual loss of 38.8 days (range 2 - 70 days). At an average follow up of 10 months (7 – 14 months) the acuity improved in five patients (83.3%) and remained stable in one (16.7%) and ranged from 20/64 to 20/200. A 2-line improvement was seen in three patients (50%). Post-operatively, the patch graft appeared flat and had a brown furry aspect in four patients and in these four patients fixation was demonstrated on the patch graft. Revascularization was visible on fluorescein and indocyanine angiography in three patients however it was not possible to determine whether perfusion of the graft was secondary to contact of the graft to remaining recipient vessels or due to in-growth of new vessels, with a possible trophic effect of the graft on the recipient bed. RPE autofluorescence also revealed almost normal macular intensity over the patch graft after excitation with a confocal scanning laser with an Argon (488 nm) beam in four patients. This indicated the presence of lipofuscin from the normal turnover of photoreceptor outer segments by RPE strongly suggesting normal interaction between photoreceptors and the RPE. The study demonstrated that transplantation of an autologous full-thickness patch of mid peripheral RPE to the macula following CNV extraction was technically feasible. Furthermore the graft was able to revascularise, remain unfibrosed, survive to be functional beyond a year, and that mid peripheral was capable of supporting foveal function.

A follow up study was performed by van Meurs et al (2006) to evaluate long term visual outcome for autologous mid peripheral full thickness RPE transplantation and to identify pre-operative factors that correlate with vision after one year (van Meurs et al., 2006). A consecutive series of 58 patients with neovascular AMD underwent surgery as outlined above. In terms of modifications to the surgical technique,

following the initial full-thickness choroidal incision to fashion the graft, a long spatula was used to sweep under the patch prior to cutting the rest of the graft free. This was performed in order to release any attachments between the choroid and the sclera, and so allow free release of the patch graft prior to cutting the reminder of the graft. The best corrected Early Treatment Diabetic Retinopathy Study (ETDRS) vision was measured pre-operatively and at one (n=58) and two years (n=19 of 58) post-operatively and converted to a logarithm of minimal angle of resolution (logMAR) score. 52 of these patients had pre-operative imaging, including colour images and angiography. The correlations between pre-operative vision, lesion type (predominantly or minimally classic, occult or haemorrhagic), size (in disc areas) of the lesion, size of sub-retinal hemorrhage, duration of visual loss and ETDRS vision at 12 months were tested with uni– and multivariate analysis. The mean pre-operative vision was 0,96 logMAR and post-operatively this mean showed a moderate improvement at 1 year to 0,90 logMAR and at 2 years to 0,77 logMAR. Pre-operative vision was 20/80 or better in 2 patients (3.4%) and after 1 year 20/80 or better in 14 (24.1%). At the 2 year point, of the 19 patients still being followed up, 4 had visions of 20/80 or better. The duration of visual loss ranged from 3 weeks to 11 months, with a median of 5 weeks and univariate analysis showed an inverse relation between duration of visual loss and vision at 12 months. Furthermore, in patients with a visual loss of less than 5 weeks duration, the size of the lesion and the size of hemorrhage were inversely related to vision at 12 months. Multivariate analysis, however, failed to confirm these associations. Post-operative complications included PVR retinal detachment in 4 patients (6.9%), suprachoroidal hemorrhage in 1 patient (1.7%) and a recurrent or persistent choroidal neovascularisation in 9 patients (15.5%). The study further confirmed that autologous full thickness mid peripheral RPE patch grafting was capable of not only stabilizing visual loss but also recovering lost function. No pre-operative factors were identified for improved patient selection, possibly due to other variables, such as the per-operative limitations of the technique mentioned above and the ^{post}-operative complications.

The next major study to evaluate this technique was performed by Joussen et al (2006) (Joussen et al., 2006). This group treated 45 eyes of 43 patients with AMD (n = 42 neovascular membranes {PED 16; occult 14}, n = 3 geographic atrophy). Pre-operative distant visual acuity ranged from 20/800 to 20/40 and reading vision ranged from 1.4 logarithm of reading acuity determination (logRAD) to 0.5 logRAD (0.04 to 0.32 Snellen equivalent). Revision surgery was required in 22 eyes as a

result of PVR, retinal detachment, macular pucker, or vitreous hemorrhage and in eight patients the patch was renewed. Post-operatively, at six months, distant visual acuity ranged from light perception to 20/50 and reading vision ranged from 1.4 to 0.4 logRAD. The distance vision improved in 11 patients (24.4%), remained stable in 5 (11.1%) and declined in 29 (64.4.%), with an increase of 15 letters in 4 eyes (9%). The results revealed the visual outcome was unrelated to the type of AMD. The angiographic studies demonstrated vascularisation of the patch graft in 40 of 42 eyes. Furthermore, in the majority of patients, autofluorescence of the pigment epithelium was coincident with revascularization of the graft. Finally, fixation on the patch graft was positively related to visual acuity and it was postulated that pre-operative fixation stability and microperimetry would assist selecting patients who would benefit from this surgical treatment. The series provided further evidence that autologous full-thickness transplantation of the choroid and RPE resulted in a vascularised and functioning graft that could lead to improved vision. The study was also the first to treat non-exudative disease and showed that vascularisation was also achieved in patients with geographic atrophy.

Thereafter a number of studies were undertaken further examining autologous full thickness peripheral RPE patch grafts widening the scope to treat exudative and non-exudative AMD with evolution to the technique. Fawzy et al (2006) reported on a consecutive series of 25 patients with neovascular AMD (sub-macular haemorrhage (5), occult only CNV (11), classic only CNV (2), mixed CNV (1) PED (4) and rupture of RPE (2)) treated with this method of RPE grafting (Fawzy et al., 2006). The study group comprised 2 eyes with classic membranes, 11 eyes with occult membranes, 1 eye with mixed membranes, 4 eyes with PED, 2 eyes with rupture of the RPE and 5 eyes with massive sub-retinal bleeding were included and all patients were evaluated prior to patch and at 6 months and 1 year follow–up. The pre-operative acuity ranged from 0.3 to 1.4 (logMAR) and at 1 year post–operative vision ranged from 0.10 to 2.10, with stabilization (± 1 line) in 5 (20%) eyes and an increase of two or more lines in 5 eyes (20%). Decrease of visual acuity was seen in most cases within the first 3 months after surgery and was mainly due to chorioretinal trauma or bleeding in 6 cases (24%), decentration of the patch in 3 cases (12%), intra-vitreal haemorrhage, lack of vascularisation of the graft, epiretinal membrane formation and cataract each in 1 eye (4%). 5 cases (20%) showed recurrence of CNV at the edge of the patch at 6 months or one year follow–up visit and were treated with laser coagulation. From 3 months to 1 year follow–up, 19 of 25 eyes showed no change (± 1 line) or an

increase of visual acuity (mean change from 3 to 12 months: increase of 0.1 logMAR). Angiography demonstrated that in patients who demonstrated vascularisation of the graft after 3 months, this persisted up to 12 months. Similarly, there was no difference in autofluorescence of the graft between 6 months and 12 months post-operatively. Microperimetry demonstrated that in patients with stable fixation at 6 months, this was maintained up to 12 months. As per the study by Joussen et al (2006), this study showed that at 1 year the surgery resulted in a viable graft and that improved vision was possible, even during the 6 to 12 month follow up period. In terms of graft viability, once vascularisation of the graft was established it persisted throughout the 12 months observation period with no evidence of graft failure. However, once again the post-operative complications were considerable including sub-retinal haemorrhage with developing fibrosis or PVR occurring within the first 3–6 months.

Kirchhof et al (2006) evaluated the technique for patients with geographic atrophy. In this study 18 patients were treated with a follow up of at least six months in 11 patients (Kirchhof et al., 2006). In the absence of removing CNV, during the technique, Bruch's membrane was scrapped prior to graft delivery to promote a local inflammatory response and encourage revascularization of the graft at the host site. The pre–operative visual acuity ranged from 20/800 to 20/32 (logMAR) and reading vision ranged from no reading vision to 0.3 (logRAD). At three months, the post–operative vision ranged from 20/800 to 20/63, and at six months the vision ranged from counting fingers to 20/32 with an increase of 2 lines in 2 eyes (11.1%). Complications included revision surgery required in 7 eyes secondary to PVR. In 16 eyes, revascularization was visible on angiography as early as 3 weeks after surgery however in one of these cases vascularisation slowly appeared after 3 months. Autofluorescence of the pigment epithelium was seen in all eyes independent of the revascularization of the graft and persisted throughout the follow–up. Microperimetry and fixation analysis revealed that six eyes had unstable fixation and/or extra-foveal fixation before surgery, of which two developed predominantly stable fixation post-operatively. All eyes with pre-operative stable or predominantly stable fixation were stable or predominantly stable post-operatively. In cases with pre-operative fixation on a central RPE peninsula, fixation remained at this position throughout the follow–up. Microperimetry also showed that the retina overlaying atrophic areas demonstrated a relative scotoma that persisted after grafting with only limited recovery. Finally no recurrence of RPE atrophy was observed on the patch during the

follow–up. This study confirmed the feasibility of the technique for geographic atrophy although the short follow up period could not exclude disease recurrence or long-term graft viability.

Treumer et al (2006) (Treumer et al., 2007) further modified the technique by fashioning a circular rather than a square graft to prevent curling of the patch. In their consecutive series of 10 patients with AMD (n=9 neovascular disease {sub-macular haemorrhage (5), large occult only CNV (3), PED (1)}; n=1 geographic atrophy), who were followed up for 1 year, the mean logMAR acuity improved from 1.37 (0.7 to 1.8) to 1.24 (0.4 to 1.24). The acuity improved in seven patients (70%), remained stable in one patient (10%), and decreased in two patients (20%). Microperimetry demonstrated light sensitivity and fixation on the sheet in five cases and angiography demonstrated perfusion through the RPE-choroid graft in nine patients. Post-operative complications included retinal detachment (n = 1), epiretinal membrane formation (n = 2), and the patient with geographic atrophy developed a CNV after surgery.

The studies above demonstrate how autologous full thickness peripheral RPE grafting has evolved from the very first report by Peyman et al in 1991 to become the primary method of RPE transplantation (Table 19). The very early studies of CNV removal and the sub-macular surgical trials established that RPE replacement was a requisite for photoreceptor survival. The search for an easily accessible source of RPE free from the constraints of immunological compatibility drove the use of autologous RPE. As outlined the techniques have developed away from the use of macular flap grafts with potentially diseased RPE to the use of free macular then peripheral cell suspensions. The failure of cell suspensions to form an adequate monolayer illustrated that the reestablishment of near physiological sub-foveal architecture was required and hence the use of full thickness grafts comprising choriocapilliaris-Bruch's-RPE.

Table 19 Landmark Studies of Human RPE Transplantation

Study	Year	No. Patients	Pathology	Graft Classification	RPE Source	Location of RPE Source	Vascular Supply	Graft Type
Peyman et al	1991	02	CNV	Autologous Homologous	Adult	Macular	Pedicle Free	Choroid RPE Patch
Algerve et al	1994	05	CNV	Homologous	Foetal	Cultured *	Free	RPE Sheet
Del Piore et al	2001	01	CNV	Homologous	Adult	Harvested **	Free	RPE Sheet
Stanga et al	2001	06	CNV	Autologous	Adult	Macular	Pedicle (1) Free (4)	Choroid RPE Patch
Binder et al	2002	14	CNV	Autologous	Adult	Peripheral (Nasal Retina†)	Free	RPE Suspension
van Meurs and Biesen	2003	06	CNV	Autologous	Adult	Peripheral (Mid Peripheral)	Free	Choroid RPE Patch

* Cultured – Foetal RPE Cells Cultured To Form A Monolayer
** Harvested – Sheets of RPE Harvested From Cadavers
† Nasal Reina – RPE Harvested Nasal to Optic Disc

4 Complications

A number of complications have been reported in the studies of human transplantation detailed above. As with macular translocation these have compromised the outcome of the surgery. Unlike full macular translocation, whereby the core surgical technique has remained unchanged since its first description, with evolutions to the surgery designed to address the complication rates, transplantation surgery has only relatively recently settled on the method of transplantation first described by van Meurs and Biesen (2003). With on going investigations, this technique has yet to undergo further scrutiny and modification.

Considering the human studies, no intra or post-operative complications were reported in the first report by Peyman et al (1991) utilizing the open sky technique of transplantation, however this was only a single case report of adult homologous and autologous transplantation. Algerve et al (1994) were the first to report the current method of graft delivery (rolled monolayer patch) through the small retinotomy created for CNV removal. In this series (homologous foetal RPE patch, n=5) again no intra-operative complications were reported, however cystoid macular oedema was reported 3 months post transplantation. Similarly a later series by this group (Algerve et al, 1997), transplanting both foetal RPE patches (n=5,exudative AMD), and cell suspensions (n=4,geographic atrophy), reported no intra-operative complications but again reported cystoid macular oedema in cases of exudative AMD, occurring with 1-6 months. Likewise, Weisz et al (1999) (homologous foetal RPE cell suspension, geographic atrophy) and Del Piore (2001) (homologous adult RPE patch, exudative AMD) both reported no complications other than a progressive rejection, in their single case reports. These early reports established that sub-retinal delivery of RPE through a small para-foveal retinotomy was relatively straightforward and the late complications were secondary to immunological rejection, a problem inherent with homologous transplantation.

In subsequent series of autologous transplantation, with development of the technique and increasing numbers treated, a small number of intra-operative complications were reported (Table 20). Stanga et al (2001, 2002) et al (autologous macular RPE patch, n=9) reported two cases in which the patch was not located at

the desired sub retinal position, and one where the patch was inverted. van Meurs et al. (2004) (autologous peripheral RPE suspension, n=8) reported two cases of RPE reflux into the vitreous cavity on cell, and one case of an intra-operative inferior RD requiring silicone oil secondary to failure of the pre-operative laser around the donor site. In all subsequent series involving autologous peripheral patch grafts (n=166), there have been a relatively small number of intra-operative complications. These include a fibrous fold across the graft (van Meurs and Biesen, 2003, n=6), two folded patches van Meurs et al, 2006, n=58), one inverted grafts (Joussen et al, 2006), ten sub-retinal haemorrhages at the donor site, peripheral retinal break, and four macular hole formation during CNV extraction (Joussen et al, 2006, n=45).

In addition to the late complications associated with homologous grafting mentioned above, a number of post-operative complications have been reported for autologous graft studies (Table 21). The occurrence of haemorrhage, particularly sub-retinal, has a profound effect on graft success and vision secondary to the direct toxic effects of the blood, the graft associated fibrosis that the blood induces, and the anatomical disruption of the photoreceptor-graft-choroidal axis. In the immediate post-operative period massive sub-retinal hemorrhage was reported in 40% of patients by Joussen et al (2006). This study reported that the larger the haemorrhage the poorer the visual outcome, with clearance of the blood and re-grafting required for very large bleeds. Furthermore, sub-retinal haemorrhage was found to be more common in patients on antiplatelet therapy and with occult CNV. Late post-operative haemorrhage was also reported by van Meurs and Biesen (2003), however this was found to resolve spontaneously and was not related to CNV recurrence and Fawzy et al (2006) also reported sub-retinal haemorrhage in 6 of 25 cases (24%). Suprachoroidal haemorrhage and vitreous haemorrhage were also reported, with one case of the former encountered each by van Meurs et al (2003) and Joussen et al (2006), and 16 cases of the later by Binder et al (2004) (14), Joussen et al (2006) (1), and Fawzy et al (2006) (1).

PVR detachments are a serious sight threatening complication in vitreoretinal surgery and much effort is undertaken to minimize this complication. The techniques of RPE transplantation themselves pose a potential risk by exposing RPE cells to the vitreous cavity, both at the time of CNV removal, as well as at the graft donor and host site during graft harvesting, delivery, sub-retinal manipulation, and post-

operatively. These factors are of particular concern with the autologous peripheral patch graft technique, as PVR detachments were not reported with homologous grafting. This was likely due to a combination of graft delivery through a very small retinotomy via a micropipette minimizing RPE reflux and the small numbers in these studies. Among the studies of autologous transplantation, the rates of PVR detachment are summarized in Table 21. Stanga et al (2001, 2002) reported 1 case (11.1%) of a total PVR detachment in this series of 9 patients treated with a macular patch graft. Using the same technique, the single case reported by Angunawela et al (2005) was also complicated by a PVR detachment. With the use of RPE cell suspensions Binder et al (2002) reported no post-operative complications, however in their second study (2004) 4 of 39 patients (10.3%) developed PVR detachments secondary to reopening of the retinotomy. Using this technique, van Meurs et al (2004) reported 3 cases of PVR in 8 patients (37.5%). Autologous peripheral full thickness patches also showed a high rate of PVR, with this complication reported in 4 of the 58 patients (6.9%) in the second series by van Meurs et al (2006), in 17 (PVR) + 11(epiretinal membrane (ERM) – a form of PVR) of 45 patients (38.8% + 24.4% respectively) by Joussen et al (2006), in 1 patient (ERM) of 25 patients (4%) by Fawzy et al (2006), in 7 patients of 18 patients (38.9%) by Kirchhof et al (2006), and in 1 (PVR) + 2 (ERM) of 10 patients (10% and 20% respectively) by Treumer et al (2006).

Measures to tackle the formation of PVR include the use of prophylactic 5-fluoro-uracil (Asaria et al, 2001) (Asaria et al., 2001), minimizing the size of and trauma to the para-macular retinotomy, and ensuring good chorioretinal adhesion around the retinotomy with minimal sub-retinal fluid post graft insertion. PVR is also minimized by as complete a removal of released RPE cells as possible and an extensive a vitrectomy as possible. The size and position of the donor site may also affect PVR rates as a posterior site may cause PVR traction on the retinotomy. Alternatively, an anterior location may result in PVR related anterior base contraction with traction on the donor site. Hence the mid peripheral site preferred utilized the investigations to date.

Other late complications reported include serous macular detachment and macular oedema in the absence of a neovascular membrane (van Meurs and Van Den Biesen, 2003; Joussen et al., 2006). Finally, CNV recurrence is a major drawback to

the success of transplantation resulting in graft failure from secondary macular oedema, sub-retinal haemorrhage and fibrosis. CNV recurrence has been reported in 9 of 58 patients (15.5%) (van Meurs et al, 2006), in 5 of 25 patients (20%) (Fawzy et al, 2006), and in the one patient with atrophic AMD (10%) (Treumer et al, 2006). In all these studies the advent of anti-VEGF agents has provided a means of treating this complication without further surgical intervention, however the long-term impact of inhibiting neovascularisation of graft vascularity is not known.

Table 20 Autologous RPE Transplantation: Intra-operative Complications

Study	Year	No. Patients	Technique	Complication Rate	
Stanga et al	2002	09	Autologous Macular RPE Patch	Incorrect Graft Position Inverted Patch	22.2% 11.1%
van Meurs et al	2004	08	Autologous Macular RPE Suspensions	Inferior RD	12.5%
van Meurs and Biesen	2003	06	Autologous Peripheral RPE Patch	Fibrous Graft Fold	16.7%
van Meurs et al	2006	58	Autologous Peripheral RPE Patch	Folded Patches	3.4%
Joussen et al	2006	45	Autologous Peripheral RPE Patch	Inverted Graft Sub-retinal Haemorrhage Peripheral Retinal Break Macular Holes – CNV Extraction	2.2% 22.2% 2.2% 8.9%

Table 21 Autologous Transplantation: Late Post-operative Complications

Study	Year	No. Patients	PVR %	ERM %	Sub-retinal Hmge %	Intraocular Hmge %	CNV Recurrence %	Other %
Stanga et al	2002	09	11					
Angunawela et al	2005	01	100					
Binder et al	2004	39	10			41		
van Meurs et al	2004	08	38					
van Meurs and Biesen	2003	06	0					17 Suprachoroidal Hmge 17 Macular Oedema 17 Serous Detachment
van Meurs et al	2006	58	07				16	
Joussen et al	2006	45	39	24	40	02		02 Suprachoroidal Hmge
Fawzy et al	2006	25	0	04	24	04	20	
Kirchhof et al	2006	18	39					
Treumer et al	2006	10	10	20				

5 Effectiveness

The effectiveness of RPE transplantation is ultimately gauged by the ability to restore stable central vision. The surgery aims to achieve this by reestablishing the defect created in the sub-foveal anatomy following CNV excision. The anatomical outcome can be monitored in a straightforward manner by the use of OCT (MacLaren et al 2005,) to examine the photoreceptor-RPE-choroid axis, and angiography to examine the vascular supply. In terms of anatomical outcome, the studies described in the previous sections demonstrate that of the transplantation strategies, only patch grafting successfully provides a continuous monolayer of RPE cells, that are correctly orientated to interact with the photoreceptor layer above, and if resting on their natural substrate, namely Bruch's membrane-choriocapilliaris, are able to reestablish an adequate blood supply. In terms of functional outcome, a number of parameters have been used to ascertain the success of transplantation. These include, visual outcomes (distance and near), autofluorescence, microperimetry, and electrodiagnositics.

Visual Outcomes

The visual outcomes for the autologous transplantation studies are outlined in Tables 22-24. These are considered below over homologous studies because, as previously mentioned, the outcomes with homologous transplantation are confounded by a number of additional variables including, the rejection response, the difficulties of RPE cell harvesting and preservation, RPE cell loss during transfer, and the absence of an adequate physiological substrate or replacement for Bruch's membrane or the choriocapilliaris. In terms of visual outcomes, autologous transplantation studies have demonstrated only modest gains of vision with relatively high complication rates significantly influencing the outcomes (Tables 22-24). Furthermore, quality of life measures have failed to correlate with visual improvement following autologous peripheral full thickness RPE transplantation.

Distance Acuity

The distance acuity outcomes for have been detailed in previous sections. Examining outcomes by series and method of transplantation, rather than individual case reports, reveals that for macular patch grafts the studies by Stanga et al (2001, 2002) (n=9) recorded at a mean follow up of 19.7 months, an improvement in distance acuity in 11.1%, with 11% improving 2-lines or more, and vision stabilized in 66.7%. The PVR rate in this series was 11.1%. The long-term follow up (McLaren et al, 2005) however revealed that despite transplantation, photoreceptor loss continued in the face of some graft survival, a finding that cast doubt on the ability of diseased macular RPE to maintain or recover lost function.

For transplantation utilising RPE suspensions, Binder et al (2002, 2004) in their pilot study (n=14) and subsequent series (n=39) at 12 months recorded distance visual and 2-line improvements both of 57.1%, with vision being stabilized in 35.7% and 30.8% respectively. The PVR rates in these series were 0% and 10% respectively, with intraocular haemorrhage occurring in 41% in the second study. A subsequent series by van Meurs, (2004) (n=8) was unable to repeat these results. In this study at a mean follow up of 11 months, vision improved in 12.5% with no patients improving 2-lines or more, and vision being stabilized in 62.5%. The PVR in the study was 38%. The rates of visual stabilization appeared impressive however closer inspection revealed the pre-operative acuity was poor and the visual gains modest relative to the severe complications encountered. The technique was subsequently abandoned in favour of full thickness patch grafts harvested from the mid periphery.

Since the original study by van Meurs and Biesen (2003), a number of investigators have reported on this technique. Examining these series reveals the reported improvement in vision has ranged from 11.1% - 83 %, with a 2-line gain reported in the range 9 – 50%, and stabilization of vision in 11 – 50%. For this technique the PVR rate has ranged from 0 – 39% with sub-retinal haemorrhage reported intra-operatively in 22% (Joussen et al, 2006) and post-operatively in 24% (Fawzy et al, 2006) and 40% (Joussen et al, 2006). Furthermore CNV recurrence has been documented in 16% (van Meurs et al, 2006) and 20% (Fawzy et al, 2006). As with macular translocation surgery the outcomes are limited by the timing and incidence of complications. This can be influenced by a number of factors including the surgical learning curve and the continued refinement of the technique.

Near Acuity

Very few transplantation studies have investigated the recovery of near function and reading (Table 23). Using Jaeger charts, Binder et al (2002) reported 21.4% (Jaeger Types 5 - 11) pre-operative reading ability. Post-operatively this increased to 85.7% however the range of Jaeger Types was much broader (Jaeger Types 1 – 16), although 7 patients (50%) achieved acuity in the range Jaeger Types 1 – 10. The subsequent study by this group failed to repeat this with only 10% achieving acuity in the range Jaeger Types 1 – 10.

In contrast, Joussen et al (2006) used the Radner reading chart and reported a decrease in reading ability from 31% (logRad 0.5 – 1.4) pre-operatively to 7% (logRad 0.4 – 1.4) post-operatively, however this was closely correlated to the occurrence of complications.

Functional and Anatomical Outcomes

Several studies have investigated graft vascularisation (angiography), retinal sensitivity (10-2 microperimetry; SLO fundus perimeter), fixation analysis (target of OCT, cross-pattern fixation target and single-point flashing light, SLO fundus perimetry, and Nidek Micro Perimetry 1), and autofluorescence patterns (SLO autofluorescence) over areas of RPE transplantation. It would be expected that good anatomical restoration would correlate strongly with functional recovery. Considering the larger series, fixation over the graft was reported in the range 21.4 – 77.8%, graft autofluorescence in the range 66.7 – 100%, and graft vascularisation in the range 50.0 – 95.2 % (Table 23).

Graft vascularisation suggests successful integration and autofluorescence and retinal sensitivity over a graft suggest a viable RPE bed supporting the overlying neurosensory retina. Examining the data no obvious pattern is apparent between these parameters however this may be due to the heterogeneity of the studies, including methods of measurements, duration of symptoms, presenting pathology, and follow up. It is important to note that these parameters may not always be associated. MacLaren et al (2005) demonstrated the loss of autofluorescence in the presence of vascularisation in their long term follow up, suggesting late RPE

/photoreceptor failure. Also autofluorescence may still be detected in the absence of vascularisation, in failing RPE cells, in the early stages of graft failure (Treumer et al, 2006). Nevertheless, these studies have provided strong evidence associating anatomical with functional restoration, as well as an insight into the mechanisms of early and late graft failure.

Analysing the results of fixation and retinal sensitivity, the 77.8% fixation documented in the series by Stanga et al (2002), was completely lost in the long-term follow up cohort (McLaren et al, 2005) despite a vascularised graft suggesting, as mentioned above, RPE/photoreceptor failure. Joussen et al (2006) reported fixation over the graft in 47.5% and showed that eyes with stable pre-operative fixation were more likely to maintain fixation post-operatively. A weak correlation between visual acuity, fixation locus, and stability was demonstrated. This study also showed that retinal sensitivity was present over grafted areas and absent over fibrosis, haemorrhage, atrophy, and non-autofluorescence. Kirchhof et al (2006) reported fixation over the graft in 77.8% of patient in their series and also demonstrated that 6 patients with unstable pre-operative fixation had recovered fixation pre-operatively. Finally, Treumer et al (2006) demonstrated the absence of fixation at 3 months post-operatively was associated with a lack of fixation at 12 months follow up.

Graft revascularization and integration, has been extensively studied with angiography for full thickness grafts, high-speed indocyanine green angiography has demonstrated choroidal and choriocapillary perfusion. Joussen et al (2006) and Truemer et al (2206) reported early graft perfusion from 1 to 16 weeks after surgery. Fawzy et al (2006) and Joussen et al (2006) both reported that once early graft vascularisation was established, it persisted for virtually all patients throughout the follow up period in each study. Comparing the three series treating geographic atrophy (Joussen et al, 2006; Kirchhof et al, 2006, Treumer et al, 2006) all reported similar revascularization rates between exudative and non-exudative disease. In terms of the mechanism of revascularization, Joussen et al (2006) also reported perfusion from the underlying choroid and their serial angiography suggested the initial vascular contact underwent later remodeling.

The studies to date suggest that RPE transplantation is a technically feasible procedure that is capable of restoring the choriocapilliaris-Bruch's-RPE-photoreceptor axis and providing support to the neurosensory retina. As with 360-degree macular translocation, the current technique of autologous transplantation of mid peripheral RPE makes it an ideal donor as the tissue is not affected by macular disease, has intact Bruch's and choriocapilliaris beneath it, and avoids the problems of rejection. The surgical technique has evolved from the first report however despite a number of advances, the recovery of function in neovascular AMD remains inconsistent and limited by a high rate of sight threatening complications. Nevertheless, the procedure, which is technically more straightforward than full translocation, offers a reconstructive treatment when neovascular disease leads to significant anatomical disruption not amenable to current treatments. Furthermore, full thickness autologous RPE transplantation may also provide a potential treatment for non-exudative disease, other macular disorders associated with RPE dysfunction, and a potential vehicle for sub-retinal delivery of therapeutic agents. This thesis will consider the surgical aspects of this treatment, the pre-operative factors influencing the outcomes, and the anatomical and functional outcomes in the context of neovascular AMD, as well as its potential for treatment beyond AMD.

Table 22 Autologous Transplantation: Distance Acuity Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre Op Vision	Post Op Vision	Improved %	Stabilized %	Declined %	Improving 2 - Lines %
Peyman et al	1991	01	CNV	14	CF	20/40	100	0	100	100
Stanga et al	2002	09	CNV	19.7	CF – 20/100	HM – 20/100	11.1	66.7	22.2	11.1
Angunawela et al	2005	01	CNV	48	CF	CF	0	100	0	0
Binder et al	2002	14	CNV	12	CF – 20/50	20/400 –20/25	57.1	35.7	7.1	57.1
Binder et al	2004	39	CNV	12	1.32*	1.11*	53.8	30.8	15.4	53.8
van Meurs et al	2004	08	CNV	11	CF – 20/400	HM – 20/400	12.5	62.5	25.0	0
van Meurs and Biesen	2003	06	CNV	10	20/200-20/400	20/64 - 20/200	83.0	17.0	0	50
van Meurs et al	2006	58	CNV	12	0.96*	0.90*	20.7	NA	NA	NA
Joussen et al	2006	45	CNV 42 GA 03	06	20/40-20/800	20/50 - PL	24.4	11.1	64.4	9
Fawzy et al	2006	25	CNV	12	0.3 – 1.4*	0.1 – 2.1*	20	20	60	20
Kirchhof et al	2006	18	GA	06	20/32 - 20/800	20/32 - CF	11.1	50.0	38.9	11
Treumer et al	2006	10	CNV 09 GA 01	12	1.37* (0.7 – 1.8)	1.24* (0.4 –1.24)	70	10	20	NA

- logMAR Acuity Mean
- NA Data Not Available/Reported

Table 23 Autologous Transplantation: Near Acuity, Functional, and Anatomical Outcomes

Study	Year	No. Patients	Pathology	Mean Follow Up /Months	Pre Op Reading Acuity	Post Op Reading Acuity	Fixation Over Graft %	Auto-fluorescence %	Vascularised %
Peyman et al	1991	01	CNV	14			100		
Stanga et al	2002	09	CNV	19.7			77.8		
Angunawela et al	2005	01	CNV	48					100
Binder et al	2002	14	CNV	12	21.4 % (Jg 5 – 11)	50 % (Jg 1 – 10)	21.4		
Binder et al	2004	39	CNV	12		10 % (Jg 1 – 10)			
van Meurs et al	2004	08	CNV	11			0		
van Meurs and Biesen	2003	06	CNV	10			66.7	66.7	50
van Meurs et al	2006	58	CNV	12					
Joussen et al	2006	45	CNV 42 GA 03	06	31 % 0.5 – 1.4*	7 % 0.4 – 1.4*	47.5†	80††	95.2†††
Fawzy et al	2006	25	CNV	12				93	
Kirchhof et al	2006	18	GA	06			77.8	100	88.9
Treumer et al	2006	10	CNV 09 GA 01	12			50	100	90

Table 23 Autologous Transplantation: Near Acuity, Functional, and Anatomical Outcomes

*	Logarithm of Reading Ability
Jg	Jaeger Reading Acuity
†	Fixation Assessed In 40 Patients
††	Autofluorescence Examined in 30 Patients
†††	Vascularisation Examined in 42 Patients

Table 24 Autologous Transplantation: Distance Visual Outcomes and Proliferative Vitreoretinopathy Rates

Study	Year	No. Patients	PVR	Improving 2 - Lines %	Improved %	Stabilized %	Declined %
Peyman et al	1991	01	0	100	100	0	100
Stanga et al	2002	09	11	11.1	11.1	66.7	22.2
Angunawela et al	2005	01	100	0	0	100	0
Binder et al	2002	14	0	57.1	57.1	35.7	7.1
Binder et al	2004	39	10	53.8	53.8	30.8	15.4
van Meurs et al	2004	08	38	0	12.5	62.5	25.0
van Meurs and Biesen	2003	06	0	50	83.0	17.0	0
van Meurs et al	2006	58	07	NA	20.7	NA	NA
Joussen et al	2006	45	39	9	24.4	11.1	64.4
Fawzy et al	2006	25	0	20	20	20	60
Kirchhof et al	2006	18	39	11	11.1	50.0	38.9
Treumer et al	2006	10	10	NA	70	10	20

* logMAR Acuity Mean/Range
NA Data Not Available

II General Methods and Materials

I Ethical Declaration

All studies fulfilled the regulation requirements of the Moorfields Eye Hospital and Whittington Hospital's Ethics and Research Governance Committees. These committees regulate research according to the United Kingdom Department of Health Research Governance protocols and the recommendations of the 1998 UK Data Protection Act. All research presented conformed to the tenets of the updated 2000 Declaration of Helsinki.

II Ophthalmic Assessment

All patients underwent a complete ophthalmic assessment at day 1 pre-operatively and at the end of the specified trial period. The assessment consisted of: best corrected visual acuity (BCVA), near visual acuity assessment and reading speed, contrast sensitivity, slit lamp bi-microscopy, Goldman applanation tonometry, ophthalmoscopy, slit lamp assessment of foveal fixation, colour fundus photography, scanning laser ophthalmoscope autofluorescence, indocyanine green and fluorescein angiography and OCT. All clinical assessments were performed by ophthalmologists and imaging performed by trained technicians.

1 Visual Acuity (Appendix 1)

The best-corrected visual acuity was measured using a back-illuminated Lighthouse visual acuity chart at 6 metres (m) and recorded as a logarithm of minimal angle of resolution (logMAR) score.

2 Near Visual Acuity Assessment (Appendix 1)

The Minnesota Low-vision Reading (MN-READ™) Acuity Charts were used at a viewing distance of 25cm in daytime northern hemisphere lighting conditions to measure: reading acuity (smallest print that can be read without significant errors), critical print size (smallest print that can be read with maximum speed) and maximum reading speed (reading speed when not limited by print size). Reading acuity and critical print size were recorded as a logMAR scores and reading speed as words per minute.

3 Contrast Sensitivity (Appendix 1)

Contrast sensitivity was measured using the Pelli-Robson contrast sensitivity chart at 1m in daytime northern hemisphere lighting conditions. The chart consists of triplet opto types 20/60 in size, whose size remains constant throughout but whose contrast decreases both down and across the chart. Pelli-Robson scores reflect the logarithm of the contrast sensitivity of each triplet group.

4 Colour Fundus Photography and Angiography

All colour fundus photographic and angiographic images were taken and processed with a Topcon retinal camera (Topcon TRC-50IX™, Topcon Medical Systems, Inc, Paramus, NJ, USA

5 Optical Coherence Tomography (OCT)

An OCT3 scan of the posterior pole was performed on all patients (*STRATUS*OCT™ version 4.1low coherence interferometer, Carl Zeiss Meditec, Inc., Dublin, CA, USA). A single fast macular scan was performed passing through the fovea

6 Scanning Laser Ophthalmoscope (SLO) Autofluorescence

An autoflourescence scan of the posterior pole was performed on all patients with the Heidelberg confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2 (HRA2)™, Dossenheim, Germany). To amplify the autofluorescence signal, a series of at least 10 digital images were captured and aligned in the fluorescein angiograph mode (FA mode). The final scan represented the composite of the individual acquisitions after detection and correction of eye movements by using image analysis software.

7 Slit-Lamp Assessment of Fixation (Appendix 3)

A simple three step slit lamp based system was used to assess the quality of foveal fixation. Firstly an 8 x 2 millimetres (mm) slit was projected onto the posterior pole and patients were asked to fix on each corner of the slit with the slit first projected vertically and then horizontally. Secondly the slit size was reduced to 2 x 2 mm and the patient assessed to see if they could maintain foveal fixation for 2-5 seconds. Finally the same slit size was presented at three differing extra-foveal locations and the patient was assessed to see if they could re-fixate with the fovea with a single saccade. Fixation was classified as good – central, good – eccentric, poor – with macular fixation, no central fixation (Table 25).

8 Post-operative Follow Up

In addition to pre-operative and final ophthalmic assessments all patients were reviewed at day 1, day 7 and at 4 weeks following each procedure. Each visit consisted of: BCVA, near visual acuity, slit lamp bi-microscopy, Goldman applanation tonometry, ophthalmoscopy and slit lamp assessment of foveal fixation.

Table 25 Slit Lamp Assessment of the Quality of Fixation

Fixation	Slit Lamp Evaluation
Good: Central	8 x 2 mm Slit – Projects to Each Corner of Slit 2 x 2 mm Slit – Maintains Foveal Fixation for 2 – 5 s 2 x 2 mm Slit – Able to Re-fixate with Fovea with Single Saccade
Good: Eccentric	As Above but Using Extrafoveal Fixation Site
Poor: Macular	Inconsistent Fixation but Using Macula to Fixate
No Central Fixation	No Macular Fixation

II Nidek MP-1 Microperimetry and Fixation Analysis (Appendix 2)

Selected patients also underwent additional assessment of retinal sensitivity and fixation pattern using a Nidek MP1 microperimeter (Nidek Co.,Ltd., Tokyo, Japan), a full description of which is given in previous literature (Varano and Scassa, 1998) and Appendix 2. Briefly each patient was presented with the minimum fixation target size that could be perceived. Microperimetry was performed using a Goldmann V stimulus and a 4-2 threshold algorithm. The supplied Nidek MP1 software was used to generate an interpolated map of retinal sensitivity projected onto a colored fundal photograph. Prior to each assessment, patients also underwent an objective fixation analysis with the Nidek MP-1 Micro Perimeter. Using a 1º single white cross target of intensity 200 cd/m^2 patients were asked to fixate the target for 30 seconds. Identification of a retinal landmark enabled the fixation analysis to be superimposed onto a retinal image.

III Macular Translocation: Surgical and Visual Outcomes

Introduction

Macular translocation continues to be the only treatment for severe cases of wet age related macular degeneration (AMD) where there is significant mechanical disruption of the retinal architecture (large sub foveal haemorrhages and retinal pigment epithelial tears – involving 'scrolling' of the epithelium). Major changes in the treatment possibilities for exudative AMD have occurred with advances in anti-vascular endothelial growth factor (VEGF) agents. Decisions to treat in AMD have historically been dominated by the characteristics of the sub-foveal neovascular membrane (CNV). This is an appropriate concept as laser photocoagulation (Group, 1991a, Group, 1991b, Group, 1994) and photodynamic therapy (PDT) (Group, 1999, Group, 2001a, Group, 2001b) are directed at modifying the CNV. This is not as relevant to the current generation of anti-VEGF agents that are far less characteristic dependent (Michels et al., 2005, Gragoudas et al., 2004, Rosenfeld et al., 2005, Spaide et al., 2006) and is an inappropriate concept when considering macular translocation as the retina is physically separated from the underlying pathology. In this situation the health of the retina and that of the choroid-RPE at the new foveal location determine the outcome. We propose that outcome is defined by retinal neurosensory function at the time of surgery and the type of CNV is irrelevant to outcome in macular translocation.

The conceptual requirements of macular translocation (MT360)(Machemer R, 1993a, Machemer R, 1993b) are to relocate the fovea while it has good restorable function, before irreversible retinal atrophy/damage has occurred and to re-establish normal sub-foveal anatomy. Despite good results reported by several workers (Eckardt C, 1999, Toth and Freedman, 2001, Lai JC, 2002, Eckardt C, 2002, Mruthyunjaya et al., 2004), the technique has failed to live up to initial expectations with a high degree of variability in visual outcomes. Although the technical aspects of surgery have been addressed, investigations into translocation surgery, to date, have concentrated on the characteristics of the CNV and visual acuity to define inclusion and outcome. We

present a novel algorithm that is based on: 1 a simple slit-lamp test of foveal fixation that allows good fixation to be determined, and 2. the duration of time between acute visual loss and presentation/surgery.

Previously it has been shown that for MT360 good outcomes depend on good fixation (Fujikado et al., 2001, Oyagi et al., 2004). Fujikado et al (Fujikado et al., 2001) demonstrated reading ability and stability of fixation as positive predictors of visual recovery and pre-operative foveal function respectively. Similarly Oyagi et al (Oyagi et al., 2004) also demonstrated with SLO microperimetry that pre-operative foveal sensitivity and fixation stability were significantly correlated with good post-operative visual outcomes.

MT360 is a therapy that attempts to rescue foveal photoreceptors and a window of opportunity for treatment exists that corresponds to the period of viability of neurosensory cells and it follows that early intervention should result in better outcomes. As visual acuity is a functional test of the neurosensory cells, good pre-operative acuity will likely reflect good retinal function. However, poor visual acuity is not an accurate indicator of poor outcome. Instead determination of residual foveal function is the prime requirement for a rescue procedure. Our algorithm suggests the two strongest indicators of foveal function are pre-operative foveal fixation characteristics and time to presentation/surgery.

This study firstly attempts to validate our slit-lamp foveal fixation task against fixation analysis as assessed by Nidek MP-1 microperimetry. Secondly the study proceeds to examine the outcomes of the case selection algorithm for MT360 by presenting the results of the first 27 consecutive cases of sub-foveal CNV treated with MT360 selected by these entry criteria. No cases were excluded so the outcomes include the learning curve for the surgery.

Methods

Slit Lamp Assessment of Fixation

A simple five step slit lamp based system was used to assess the quality of foveal fixation as described in the General Methods.

Validation of Slit Lamp Assessment of Foveal Fixation

To validate the slit lamp assessment of foveal fixation, a cohort of patients with macular disease including patients referred for consideration for MT360 were assessed by two separate investigators and the fixation of both eyes classified as per the slit lamp assessment described above. These patients subsequently underwent objective fixation analysis with the Nidek MP-1 Micro Perimeter (Nidek Co.,Ltd., Tokyo, Japan) as described in the General Methods and Appendix 2. When fixation analysis was not possible with a 1 degree cross the analysis was performed with a 3 degrees cross. For comparison age matched patients with no macular disease also underwent two investigator slit lamp and microperimetry assessment of fixation.

Patients

A prospective series of 27 consecutive patients (17 female, 10 male) with a mean age of 76 (57 – 95) years and CNV secondary to AMD underwent MT360 with silicone oil tamponade and subsequent counter-rotation surgery at Moorfields Eye Hospital, London between May 2003 and May 2006 (Table 26). The study was conducted with full ethical approval as outlined in the General Methods.

Case Selection Algorithm: Inclusion and Exclusion Criterion

Patient selection was undertaken using an algorithm based primarily on the potential pre-operative viability of foveal photoreceptors and secondarily on the health of the retinal pigment epithelium-choroidal axis of the new foveal location. These parameters were assessed by direct and indirect methods (Table 27).

The patients were invited to take part in the macular translocation study with informed consent if they met all the following criteria:

Inclusion Criteria

- Good foveal fixation
- Short duration from loss of reading ability
- Healthy RPE-Choroidal axis at new foveal location
- Sub-foveal choroidal neovascular membrane
- AMD affecting the second eye where navigational vision remains present in the first eye
- Potential for the fovea to adequately clear the edge of the sub-retinal pathology following 45^{o} rotation

Exclusion Criteria

- Unfit for general anaesthetic
- Unwillingness to undertake follow-up
- Other ophthalmic pathology precluding complex vitreoretinal surgery
- Inability to give informed consent

Pre and Post-operative Assessment

All patients underwent a complete ophthalmic assessment at day 1 pre-operatively and at 12 months post-operatively. The assessment was performed as outlined in the General Methods.

All patients were scheduled to return at 3, 6, 9 and 12 months following translocation surgery. In addition all patients were reviewed at day 1, day 7 and at 4 weeks following each procedure and assessed as outlined in the General Methods. Prior to and following counter-rotation surgery all patients underwent a full orthoptic assessment.

Surgical Technique

All surgeries were performed under general anaesthesia by a single surgeon: MT360 (LDC); Removal of silicone oil (LDC); Counter-rotation surgery (JL/JA). The technique used for MT360 has been previously described by Eckardt and Eckardt 1999 (Eckardt C, 1999), and Toth and Freedman 2001 (Toth and Freedman, 2001). During the procedure the primary CNV was managed by removal using Eckardt forceps, and the feeding choroidal vessel coagulated with endolaser. Counter-rotation of the translocated eye was performed as described previously (Toth and Freedman, 2001).

Statistical Methods

The primary efficacy outcome was the change in: distance visual acuity (logMAR), reading acuity (logMAR), critical print size (logMAR), reading speed (words per minute), and contrast sensitivity.

The data was described by means, standard deviations and frequencies, as well as corresponding 95% confidence intervals (CI). Detailed comparisons were performed with Wilcoxon-Signed non-parametric analysis using SPSS statistical software (SPSS Inc, Illinois, USA). Effects were assessed as significant if the P value of the corresponding test fell below P 0.05.

Table 26 Patient Demographics, Duration of Symptoms, and Pre-operative Status of the CNV

Sex	No.	Mean Age / Years	Pathology	Mean Duration of Visual Loss / Weeks
Female	**17**	**75** **(57 – 95)**	**Occult 12** **SRH 5**	**8** **(03 – 12)**
Male	**10**	**78** **(61 – 88)**	**Occult 6** **SRH 4**	**9** **(04 – 32)**

SRH Sub Retinal Haemorrhage
Occult Occult Choroidal Neovascular membrane

Table 27 Algorithm for Patient Selection for Macular Translocation Surgery

Foveal Photoreceptor Viability	**Adequacy of RPE – Choroidal Interface at the Recipient Foveal Site**
• Symptom duration (loss of reading ability) of less than 3 months • History of rapid onset of visual loss at presentation • Good foveal fixation as determined by the slit lamp fixation task	• Indocyanine green and fluorescein angiography • Scanning laser ophthalmoscopy autofluorescence

Results

Validation of Slit-Lamp Analysis of Fixation

To validate the slit-lamp based analysis of fixation; two investigators on the slit- lamp and also with the Nidek MP-1 fixation analysis programme classified the fixation of a cohort of patients with macular disease and age-matched normals. There was 100% concordance between the two investigators for slit-lamp classification of fixation. 15 eyes of age-matched patients with no macular disease and a mean logMAR acuity of 0.11 (0.00 – 0.18) were all classified as having foveal fixation with the slit-lamp assessment. For this group Nidek MP-1 fixation analysis resulted in 99.8 % (99 – 100) and 100% of test time that fixation remained within 2 and 4 degrees of the fixation target respectively (Figure1).

Of 30 eyes of patients with macular disease (2 patients Geographic atrophy; 1 patient inherited macular dystrophy; 27 patients AMD) 15 were classified as having unstable fixation and 15 as having no–fixation by slit lamp assessment. Eyes classified as having unstable fixation had a mean acuity of logMAR 1.01 (0.48 – 1.62) and Nidek MP-1 analysis resulted in 51% (19 -79) and 79.5% (51 – 99) of fixation time within 2 and 4 degrees of the target respectively (Figure1). Eyes classified as no–fixation had a mean acuity of logMAR 1.56 (1.00 – 1.80) and Nidek MP-1 analysis resulted in 13.5% (06 -25) and 39.9% (14 – 58) of fixation time within 2 and 4 degrees of the target respectively. The difference in logMAR acuities and fixation points within 2 and 4 degrees, between age matched normal eyes and those eyes classified with either unstable or no–fixation were highly significant ($P<0.001$), as were the differences in these parameters between eyes with unstable and no–fixation ($P<0.001$). The results are summarised in Table 28.

Figure 1 Slit-Lamp Assessment and Nidek MP-1 Assessment of Foveal Fixation

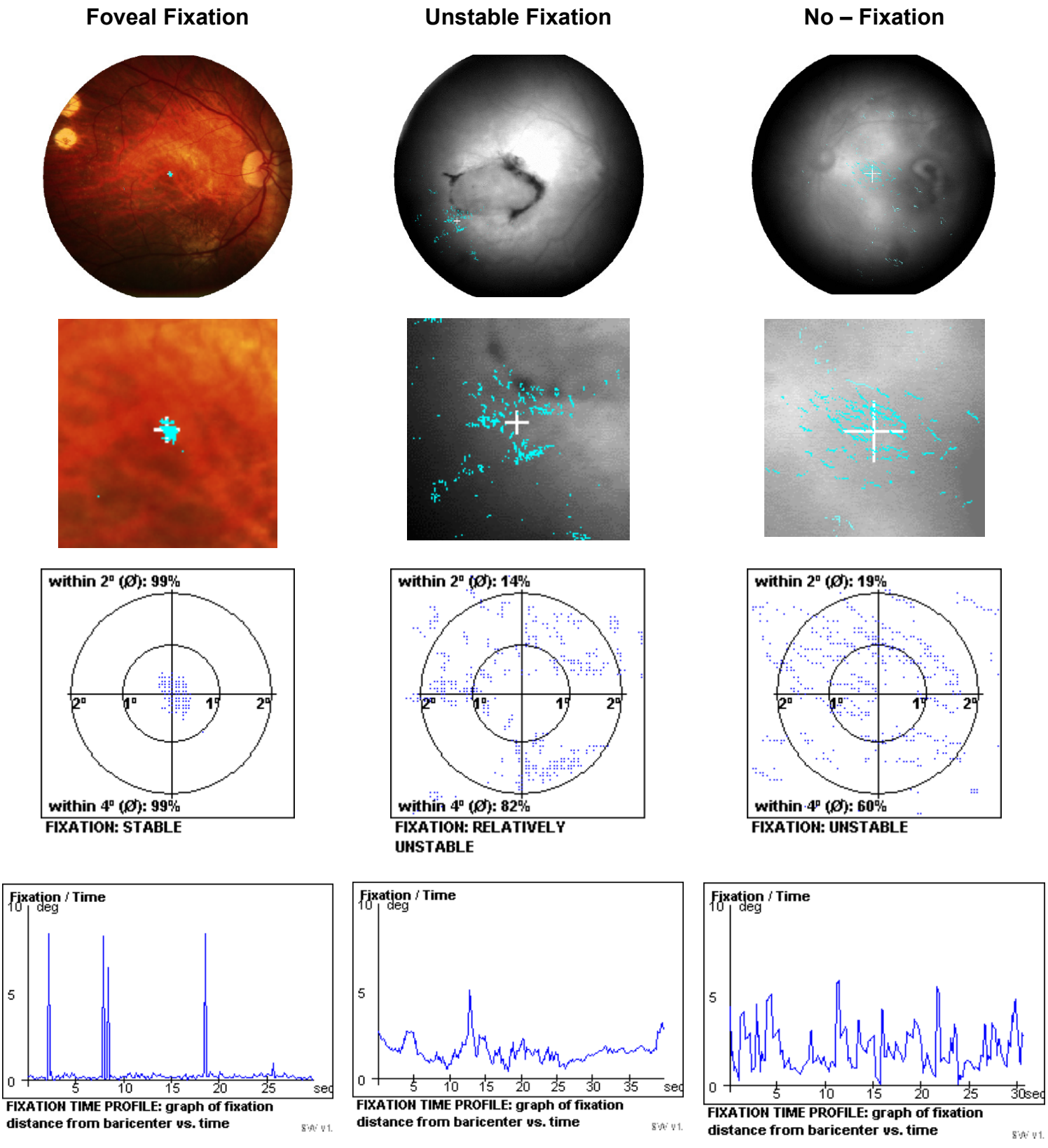


Table 28 Fixation Analysis Correlates: Slit-Lamp Classification vs. Nidek MP-1 Microperimeter

Slit Lamp Classification of Fixation	Eyes Assessed	Mean ETDRS Acuity / logMAR	Mean % Time Fixation within 2 ° of Target	Mean % Time Fixation within 4 °of Target	Nidek MP-1 Classification of Fixation
Foveal	15	0.11 (0.00 – 0.18)	99.8 (99 – 100)	100 (100 – 100)	Stable
Unstable	15	1.01 (0.50 – 1.62)	51.0 (19 – 79)	79.5 (51 – 99)	Relatively Stable
No – Fixation	15	1.56 (1.30 – 3.0)	13.5 (6 – 25)	39.9 (14 – 58)	Unstable

Visual Outcomes

Distance Acuity

The ETDRS distance acuity improved from a mean pre-operative logMAR acuity of 0.88 (0.18 – 2.10) to 0.68 (0.12 – 1.80) (P<0.03) post-operatively at an average follow up of 12.2 months (04 – 36). 66% of patients achieved a distance acuity of ≤ logMAR 0.8 (20/100), 22% distance acuity of ≤ logMAR 0.3 (20/40) and 33% gained 3 lines of visual acuity.

Reading Acuity

During the same follow up period the mean MN Read™ reading acuity improved from logMAR 1.23 (0.60 – 2.00) to 0.91 (0.22 – 2.00) (P<0.01). 44% of patients achieved a reading acuity of ≥ logMAR 0.7(N10), 15% achieved near acuity of ≥ logMAR 0.4 (N5) and 44% gained 3 lines of reading acuity. Furthermore there was a significant improvement in reading speeds from a mean pre-operative speed of 89 (0 – 176) wpm to a mean post-operative speed of 182 wpm (0 – 231) (P<0.03) and an improvement in the critical print size from a pre-operative mean of logMAR 1.26 (0.8 – 2.0) to a post-operative mean of logMAR 0.96 (0.3 – 2.0) (P<0.001). Finally the Pelli-Robson contrast sensitivity also improved from a mean pre-operative logMAR of 0.51 (0 – 1.20) to a mean post-operative logMAR of 0.92 (0 – 1.8) (P<0.01). The visual outcomes are summarised in Figure2.

Pre-operative Acuity and Post Surgical Visual Outcomes

29.6% (8/27) of patients had a pre-operative ETDRS acuity of ≥ logMAR 1.0 and this group demonstrated a mean improvement in distance acuity of logMAR 0.52 (0 – 1.68). 74.1 % (20/27) of patients had a pre-operative MNRead™ reading acuity of ≥ logMAR 1.0 and this group demonstrated a mean improvement in reading acuity of logMAR 0.31 (-0.96 – 1.36). Finally of the 22% (6/27) of patients who achieved a distance acuity of ≤ logMAR 0.3, the mean pre-operative distance acuity was 0.74 (0.18 – 1.8) (Table 29).

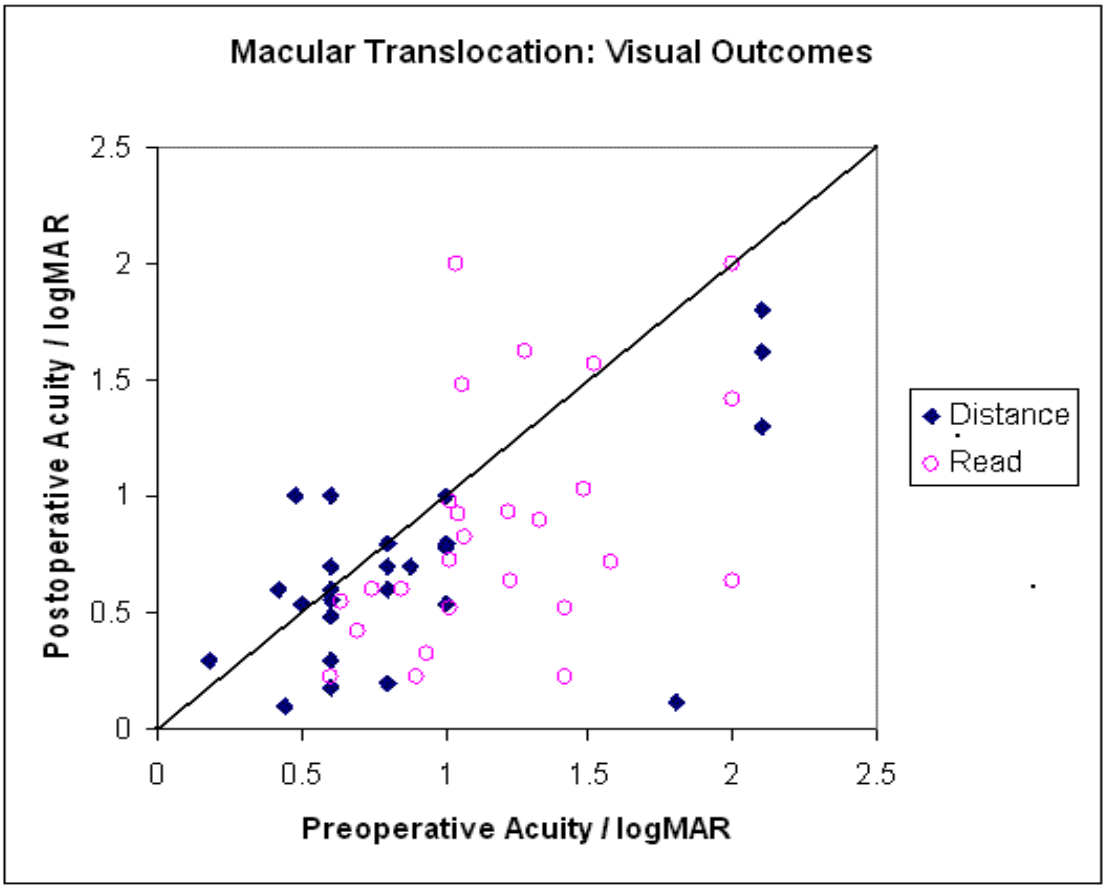
Time to Surgery

The mean time between onset of presenting visual decline and surgery was 8.4 (03 – 32) weeks. The relationship between the time to surgery and the change in logMAR acuity is illustrated in Figure 3.

Surgical Outcomes

Surgical complication rates are summarised in Table 30.

Figure 2 Pre- and Post-operative Distance and Reading Acuities



29 Pre-operative Acuity and Visual ≤ logMAR 0.3

Patient No.	**3**	**8**	**9**	**10**	**21**	**23**
Preop Distance Acuity	0.44	0.6	1.8	0.8	0.6	0.18
Postop Distance Acuity	0.1	0.3	0.12	0.2	0.18	0.3

Figure 3 Time to Surgery and Visual Outcome

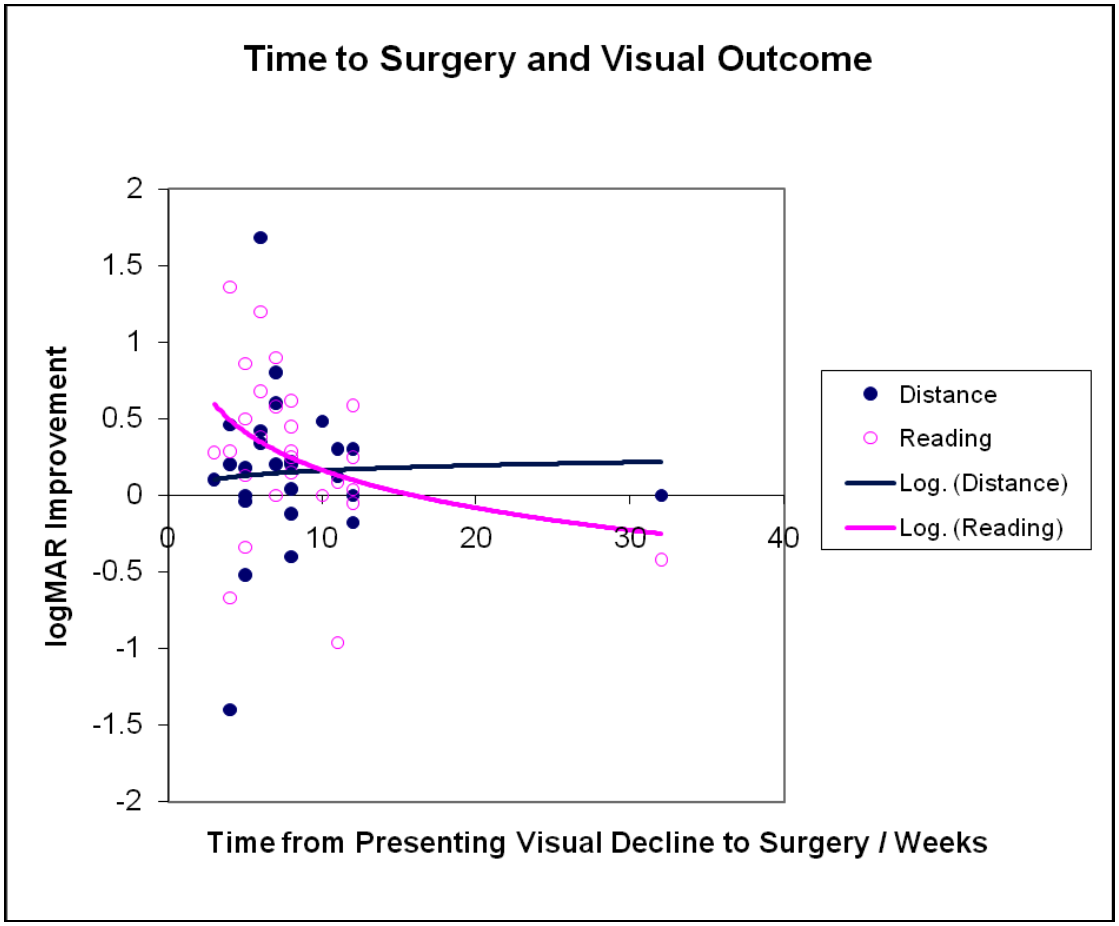


Table 30 Surgical Complications

Complication	**%**
Proliferative Vitreoretinopathy	7
Retinal Detachment	7
Macular Oedema	7
CNV Recurrence	11
Angle Closure Glaucoma	4
ERM with Pucker	4

ERM – Epiretinal Membrane

Discussion

The concepts underlying translocation surgery for the treatment of neovascular AMD are significantly different to alternate treatments directed at suppression of the choroidal new vessels. Laser photocoagulation, PDT and to a lesser extent anti-VEGF have focused on vessel characteristics while in translocation it is felt they are much less significant. Translocation does not modify the disease process but is a rescue procedure for foveal photoreceptors. As such outcome depends on macular and foveal function. Given this it is proposed that the case selection needs to be made in a completely different way, as outlined by this algorithm.

This study has proposed a clinical case selection algorithm designed to identify patients who will maximally benefit from MT360. The algorithm consists of two components – a simple slit-lamp based clinical assessment of foveal function and an estimate of the duration of acute visual loss with a time cut-off for inclusion. The slit lamp beam provides a supra - maximal stimulus that allows the fovea to be stimulated even when sub-retinal blood, fluid and the CNV are reducing photoreceptor function. With this supra-maximal stimulus the health of the fovea can be tested using the accuracy and persistence of fixation – a very powerful indicator of retinal function. The simple tasks are easily carried out by the patient and give a quick and very clear indication of the level of foveal function, which appears to be a predictor of outcome.

Estimating the duration of visual loss is based on the extrapolation that with no intervention in a case of neovascular AMD the vision becomes irreversibly damaged. Early in the disease process the retina is affected functionally and reversibly but with time the damage becomes permanent and irreversible (Green and Enger, 1993, Curcio et al., 1996). As such a critical window of opportunity exists to: 1. Salvage function from the existing photoreceptor pool before fibrovascular proliferation causes marked ‘irreversible’ photoreceptor loss 2. Treat any visual loss that may be due to secondary and potentially ‘reversible’ factors such as sub-foveal fluid and haemorrhage and 3. Mechanically restore normal anatomy. Consequently any

attempt to recover vision is more likely to succeed if early rather than late rescue is performed.

This case selection algorithm proposes a simple slit-lamp based assessment of fixation using a supra-threshold slit lamp target to assess retinal function. To validate this method of fixation analysis a comparison was made against an objective method to determine foveal fixation – the Nidek MP-1 microperimeter. Two investigators, using the slit-lamp protocol, graded foveal fixation in a cohort of patients with macular disease and in a group of age matched normals. There was 100% concordance between the two investigators and between slit-lamp and Nidek MP-1 microperimetry classification of fixation (Figure 1; Table 28). Each of the three groups, as classified with the slit-lamp, were also significantly different from each other for ETDRS acuity and for the duration of time that fixation was maintained within 2- and 4-degrees of the central target on microperimetry analysis. The results demonstrate that fixation can be assessed by a slit-lamp based method that has the advantages of being both easy to administer and accurate.

The results of the first 27 consecutive patients treated with MT360, including the surgical learning curve, as selected by the algorithm illustrate a marked restoration of distance and reading vision. At an average follow up of 12.2 months there was a significant improvement in the mean distance acuity, improving from logMAR 0.88 to 0.68 ($P<0.03$). 66% of patients achieved distance acuity of ≤ logMAR 0.8 (20/100), 22% distance acuity of ≤ logMAR 0.3 (20/40) and 33% gained 3 lines of visual acuity.

During the same follow up period a significant improvement in all the parameters of near function was observed. The mean reading acuity significantly improved from logMAR 1.23 to 0.91 ($P<0.01$). 44% of patients achieved a reading acuity of ≤ logMAR 0.7(N10) and 15% achieved reading acuity of ≤ logMAR 0.4 (N5). In addition there was a significant improvement in the mean reading speed from 89 to 182 wpm ($P<0.03$), the critical print size which improved from logMAR 1.26 to 0.96 ($P<0.001$) and the contrast sensitivity which improved from logMAR 0.51 to 0.92 ($P<0.01$). Reading is defined as fluent at > 80 words per minute (Whittaker and Lovie-Kitchin, 1993, Crossland et al., 2005b) and 74.1% achieved this following MT360.

These results compare favourably with previous investigations into MT360 (Eckardt C, 1999, Seaber JH, 1997, Toth et al., 2004, Abdel-Meguid et al., 2003, Pertile and Claes, 2002), particularly the study by Pertile and Claes (Pertile and Claes, 2002) who performed MT360 on 50 patients with neovascular CNV. In this particular study 68% of patients recovered reading vision with 58% and 18% achieving ≥ 20/100 and ≥ 20/40 distance acuity respectively. When considering case selection we feel the crucial determinants with regards to outcome in the study by Pertile and Claes, as with the present study, were a short duration of symptoms – all patients had suffered a recent drop in vision and demonstrated good residual foveal function – most patients were able to read newspaper print until 3 months before the procedure.

Subgroup analysis of patients with the best visual outcomes (post-operative distance or reading acuity of ≤ logMAR 0.3) (Patients 3, 8, 9, 10, 21, 23) revealed a mean duration of visual loss of 7.5 weeks (06 – 12). This compares with a mean duration of visual loss of 8.4 (03 – 32) weeks for the entire study group. Of further note is the finding that for this subgroup the pre-operative data suggests that while good pre-operative vision (Patient 3, 23) will likely result in a good post-operative outcome, it is healthy pre-operative neurosensory retina that is required for MT360 to be able to rescue function and not necessarily good pre-operative vision, as evidenced by Patients 8, 9, 10 and 21 (Table 29).

Subgroup analysis of patients with the worst pre-operative acuities (patients with a pre-operative distance or reading acuity of ≥ logMAR 1.0) revealed a mean improvement in distance and reading acuity of logMAR 0.52 (0 – 1.68) and 0.31 (-0.96 – 1.36) respectively. This compares with distance and reading improvements in the entire study group of logMAR 0.20 (- 0.52 – 1.68) and 0.31 (-0.96 – 1.36). These subgroup analyses further support the premise of the case selection algorithm that those patients with a short duration of acute visual loss and displaying good foveal fixation, irrespective of pre-operative acuity, should have the best chance of visual recovery following MT360.

An obvious weakness of this study is that surgery was not performed on those patients with poor visual acuity and poor fixation to objectively assess the predictive power of our algorithm. Furthermore it can be argued that duration of visual loss

could be used without assessing fixation behaviour as intuitively the two parameters are related. However simply using duration of visual loss to predict outcomes would not identify which patients were still within the critical window of opportunity to recover residual photoreceptor function. Although it is estimated this period is in the region of 12 weeks, the exact duration will depend on the number of remaining viable photoreceptors at onset of visual loss and the severity and course of the disease process. It is expected that patients with a slow insidious disease course will have a smaller window of recovery following acute loss of reading vision compared to patients with a short overall disease onset. As such fixation behaviour becomes an important determinant of the critical period of photoreceptor viability. When used together with time since acute visual loss, it is felt that both parameters become a powerful method of assessing suitability for MT360 and good outcomes. Finally, to strengthen this algorithm further and indicate the contribution of the fixation task vs. duration of visual loss, ideally an examination of cases where there is a marked discordance between fixation behaviour and duration of visual loss is required. However, as would be expected by the natural history of the disease, no cases of good fixation and a markedly extended period of visual loss were encountered, and neither were there any cases of poor fixation and immediate presentation.

The study has demonstrated a simple case selection algorithm that assesses the residual foveal function and suggests good visual outcomes. The algorithm is based on the duration between acute visual loss and surgery and foveal fixation as demonstrated by a simple slit-lamp fixation task. Thus patients with a short duration of visual loss and demonstrating good foveal fixation have a good potential for visual recovery in uncomplicated MT360. Furthermore our algorithm suggests good visual outcomes independently of pre-operative visual acuity and CNV characteristics. There was no restriction on the entrance visual acuity – the traditional method of predicting good visual outcome – as the algorithm is aimed at selecting patients even in cases of poor vision at presentation. This was demonstrated by the pre-operative visual acuity of our first 27 cases that averaged logMAR 1.22 (0.42 – 2.10). This is in contrast to previous studies where case selection has relied on either the pre-operative visual acuity and/or characteristics of the CNV, with variable outcomes. The algorithm suggests the two strongest indicators of foveal function are pre-operative foveal fixation characteristics and time to presentation/surgery. The algorithm is simple in its application and does not require any additional investigations.

IV Macular Translocation: Quality of Rescue

Introduction

Difficulty in reading is the commonest disability reported secondary to central visual loss from macular disease. The ability to read is a highly valued task and its loss is linked to considerable psychological and social distress, and a marked reduction in quality of life (McClure et al., 2000, Elliott et al., 1997, Hazel et al., 2000, Legge et al., 1985, Legge et al., 1992, Regillo et al., 2008, Rubin et al., 1994).

Exudative age related macular degeneration (wet AMD) is a common, rapid cause of central vision loss. Recent advances in anti-VEGF treatments have offered the possibility of reversal of vision loss. In severe lesions, notably where there is significant disruption of the retinal anatomy (e.g.: large sub-retinal haemorrhage or retinal pigment epithelial tear), treatments options are limited to either surgical methods designed to restore normal anatomy (macular translocation or retinal pigment epithelial transplantation) or visual rehabilitation. In terms of visual recovery, a primary goal of such treatments is the restoration of reading.

Conventional rehabilitation with low visual aids tries to restore reading by use of magnification. Despite magnification, reading speeds are often limited to < 50 words per minute, well below the level required for fluent reading (> 80 words per minute (Whittaker and Lovie-Kitchin, 1993, Crossland et al., 2005b)). Macular translocation surgery (MT360) has been used to restore reading vision in severe wet AMD. MT360 attempts to rescue foveal photoreceptors before irreversible retinal atrophy/damage has occurred, re-establish normal sub-foveal anatomy, and provide stable improved central vision.

Good visual outcomes have been reported when MT360 is performed during the window of opportunity for treatment that corresponds to the period of viability of

neurosensory cells (Eckardt C, 2002, Eckardt C, 1999, Lai JC, 2002, Pertile and Claes, 2002, Mruthyunjaya et al., 2004). In the majority of studies outcome measures have concentrated on distance acuity rather than near visual function or reading speed. Of note Eckardt (1999) (Eckardt C, 1999) and Lai et al (2002) (Lai JC, 2002) reported improvements in near acuity following translocation; Fujikado et al (2002) (Fujikado et al., 2002a) specifically examined reading ability following MT360 and reported an improvement in critical print size determined using a Japanese 1version of the Minnesota Acuity Chart (MN Read™ – J Chart); and Toth et al (2004) (Toth et al., 2004) investigated various parameters of near visual function after translocation and reported significant improvements in near acuity, contrast sensitivity and reading speed.

This study aims to demonstrate the optimal potential of MT360 in the restoration of normal visual function. An infrared eyetracker and microperimetry are used to examine reading behaviour (reading speeds and saccadic activity), foveal fixation characteristics (preferred retinal loci and stability of fixation), and retinal sensitivity (to establish the threshold visual function) at the new macular location in order to describe the quality and extent of this recovery. These examinations are performed in the best outcome cases from a consecutive series of 23 patients with sub-foveal choroidal neovascular membrane (CNV) treated with MT360. MT360 is an invasive and involved procedure (new foveal location, extraocular muscle surgery, residual torsion) and documenting the potential for restoration of close to normal function is valuable.

Methods

Patients

Six patients (3 female, 3 male) with a mean age of 69 (61 – 74) were recruited from the prospective series of 27 consecutive patients detailed in Chapter III. The six recruited to the study represented the best outcomes from the translocation surgical series (Table 31). The study was conducted with full ethical approval as outlined in the General Methods.

Inclusion Criterion

The six patients were invited to take part in the trial with informed consent if they met all the following criteria:

- A post-operative reading acuity of logMAR 0.70 or better when tested with the Minnesota Reading Acuity Chart (MN Read™) (Precision Vision, La Salle, IL, USA) to able to perform the reading and fixation tasks outlined below.

- No symptoms of diplopia or tilit

Infrared Eyetracker Assessment of Reading and Fixation

Eye Movement Recording

Eye movements were measured using the SMI Eyelink Gazetracker (SensoMotoric Instruments, Teltow, Germany) running EyeLink Software v2.04 (Appendix 4). The EyeLink gazetraker is a headband-mounted apparatus on which are mounted two adjustable infrared cameras that record eye position using the 'bright pupil' technique. A third camera on the headband tracks head motion by monitoring infrared emitters positioned at the corners a high resolution display monitor (21" Trinton GDM-F500R, Sony Corporation, Tokyo, Japan) with a resolution of 800x600 pixels and a refresh frequency of 85Hz. The luminance of the white background of the screen was 125cd/m^2. Patients were seated 50cm from the monitor and wore suitable refractive correction for the screen distance. With this arrangement

compensation for head motion was made so that a real position of gaze could be calculated. Eye position was measured at a temporal resolution of 250Hz with the manufacturers reporting a gaze position accuracy of <0.5º.

Before each assessment, eyetracker calibration, validation, and drift correction were performed using the algorithms provided for this purpose. Only trials where calibration was categorized as good by the Eyelink software were included. Saccades were defined as being eye velocity movements with velocity >30ºs^{-1} or acceleration >8000ºs^{-2} and described in terms of their number, average latency, average velocity, and direction (horizontal/vertical/forward/regressive).

Reading Assessment

A full description of the eyetracker assessment of reading is given in previous literature (Crossland et al., 2004a, Crossland and Rubin, 2006).

Assessment of Horizontal and Vertical Saccades

A full description of the eyetracker assessment of horizontal and vertical saccades is given in previous literature (Crossland et al., 2004a, Crossland and Rubin, 2006).

Assessment of Preferred Retinal Locus/Loci (PRL) and Fixation Stability

A full description of the eyetracker assessment of fixation stability is given in previous literature (Crossland et al., 2004a, Crossland and Rubin, 2006, Crossland and Rubin, 2002, Crossland et al., 2004b).

The data collected was used to describe fixation by calculating a bivariate contour ellipse as described in previous literature (Crossland et al., 2004a, Crossland et al., 2005a, Crossland and Rubin, 2006, Crossland and Rubin, 2002, Crossland et al., 2004b). A bivariate contour ellipse describes the locus of fixation in normal observers. The area of this ellipse – bivariate contour ellipse area (BCEA) – indicates fixation stability. The BCEA of normal individuals is approximately 80-1200minarc^2 (Crossland and Rubin, 2002) and with macular disease they range from near normal to over 13 000 minarc^2 (Rohrschneider et al., 1995). The use of a non-parametric modeling technique (the kernel density estimator) was used to assess if individual

BCEAs provided a better fit to the data than a global BCEA. This analysis was used to determine the number of PRL

Eyetracker Control / Comparative Data Groups

Data collected from eyetracker assessment following MT360 was compared with similar data collected from normal controls (Normal), patients with dry age related macular degeneration (Dry AMD) and patients with wet AMD (Wet AMD). Each group contained ten age-matched patients who underwent the aforementioned eyetracker assessment of reading and fixation (Table 32).

Microperimetry Assessment of Retinal Sensitivity and Fixation

All patients also underwent of post-operative assessment of retinal sensitivity and fixation pattern using the Nidek MP-1 Micro Perimeter (Nidek Co.,Ltd., Tokyo, Japan) as described in the General Methods and Appendix 2.

Statistical Methods

Descriptive statistics (n, median, range, minimum, maximum, interquartile range) were obtained for each data set (MT360 cohort and the control/comparative groups). The data sets were examined for statistical differences in ETDRS acuity, MN Read™ Reading Acuity, MN Read™ Critical Print Size, MN Read™ Critical Print Reading Speed, Pelli-Robson Contrast Sensitivity, eyetracker reading speed, horizontal and vertical saccades and saccadic speed and latency, global fixation, and number of preferred retinal loci. Statistical analysis was performed using the Mann-Whitney test on SPSS statistical software (SPSS Inc, Illinois, USA). All outcome measures quoted below represent median values unless otherwise stated.

Table 31 Eyetracker Cohort: Patient Demographics and Post-MT360 Outcomes

Patient No.	Age	Sex	Follow Up Post MT360 / Months	Residual Torsion / Degrees	ETDRS Distance Acuity / logMAR			MN Read™ Reading Acuity / logMAR			MN Read™ Critical Print Size / logMAR			MN Read™ Reading Speed / Words Per Min			Pelli-Robson Contrast Sensitivity / PR log CS		
					Pre Op	Post Op	Δ	Pre Op	Post Op	Δ	Pre Op	Post Op	Δ	Pre Op	Post Op	Δ	Pre Op	Post Op	Δ
1	74	F	29	05 (in)	0.44	0.10	**0.34**	0.60	0.22	**0.38**	1.10	0.50	**0.60**	150	231	**81**	1.05	1.65	**0.60**
2	63	F	20	10 (in)	0.60	0.30	**0.30**	0.85	0.60	**0.25**	1.10	0.90	**0.20**	146	133	**13**	0.90	1.20	**0.30**
3	69	F	16	05 (in)	1.80	0.12	**1.68**	1.42	0.22	**1.20**	1.10	0.70	**0.40**	70	177	**107**	0.00	1.65	**1.65**
4	71	M	12	03 (in)	0.80	0.20	**0.60**	1.43	0.52	**0.91**	1.20	0.50	**0.70**	52	177	**125**	0.60	1.20	**0.60**
5	74	M	05	08 (in)	0.88	0.52	**0.36**	1.02	0.52	**0.50**	1.10	1.10	0	73	55	**18**	0.30	1.55	**1.25**
6	61	M	06	00	0.50	0.60	**0.10**	1.02	0.66	**0.36**	1.10	1.10	0	140	139	**1**	1.05	1.30	**0.25**

in Incyclotorsion

Table 32 Control / Comparative Group Demographic

Group	**N**	Mean Age / Years	**Diagnosis**
Normal	10	71.0 (62 – 79)	No Pathology
Dry AMD	10	76.2 (62 – 84)	Geographic Atrophy 9 Macular Drusen 1
Wet AMD	10	79.2 (74 – 85)	CNV 7 PED 3
MT360	06	68.7 (61 – 74)	CNV 6

CNV Choroidal Neovascular Membrane
PED Pigment Epithelial Detachment

Results

Surgical Series

The surgical series of 27 patients (17 female, 10 male; aged 57 – 95 years) with sub-foveal CNV treated with MT360 illustrated a restoration of distance and reading acuity (Figure 2).

Eyetracker MT360 Cohort

The six best outcomes (3 female, 3 male; aged 61 – 74) form the aforementioned series were recruited to investigate reading behaviour post MT360 (Table 31). At 14 (5 – 29) months follow up this cohort (MT360 cohort) improved their distance acuity from logMAR 0.70 (0.44 – 1.80) to 0.25 (0.10 – 0.65) ($P>0.05$) and the reading speed increased from 106 (52 – 150) to 158 (55 – 231) wpm ($P>0.05$). In contrast, there was a significant improvement in the reading acuity, logMAR 1.02 (0.60 – 1.43) to 0.52 (0.22 – 0.66) ($P<0.01$), critical print size, logMAR 1.10 (1.10 – 1.20) to 0.80 (0.50 – 1.10) ($P<0.05$), and contrast sensitivity, logMAR 0.75 (0 – 1.05) to 1.43 (1.20 – 1.65) ($P<0.02$) (Table 33).

The relationship between distance and reading acuity, pre- and post-MT360, is summarised in Figures 4 and 5. Pre-operative distance acuity was well correlated with pre-operative reading acuity (r = + 0.71), however no such correlation was found between pre-operative distance acuity and the pre-operative critical print size (r = - 0.04) (Figure 4). Following surgery both the reading acuity and critical print size significantly improved and both parameters were well correlated with the post-operative distance acuity (Figure 5).

Comparison of Visual Function: MT360 vs. Normal vs. AMD

Distance Acuity, Reading Function, Contrast Sensitivity

Outcome measures (distance and reading acuity, critical print size, reading speed, contrast sensitivity) for the post MT360 cohort and the comparative groups are summarised in Table 34. For each measure the recovery following MT360 was

greater than both of the untreated AMD groups and less than the age matched normal group (Figures 6,7 and 8). The improvement for the MT360 cohort over the AMD groups reached significance ($P<0.05$) for all parameters except critical print size and contrast sensitivity. The results for the MT360 cohort were also significantly different ($P<0.05$) to age matched normal subjects except for reading speed (Figure 8).

Eyetracker Assessment of Reading Function

Horizontal Saccade Task

Assessment of horizontal saccades demonstrated that the MT360 cohort performed a greater number of saccades than the comparative groups, a result that reached significance ($P<0.05$) for all but the wet AMD group. The saccades were of longer latency ($P>0.05$) and of reduced velocity ($P<0.05$) (Table 35) (Figures 9,10 and 11).

Reading Task

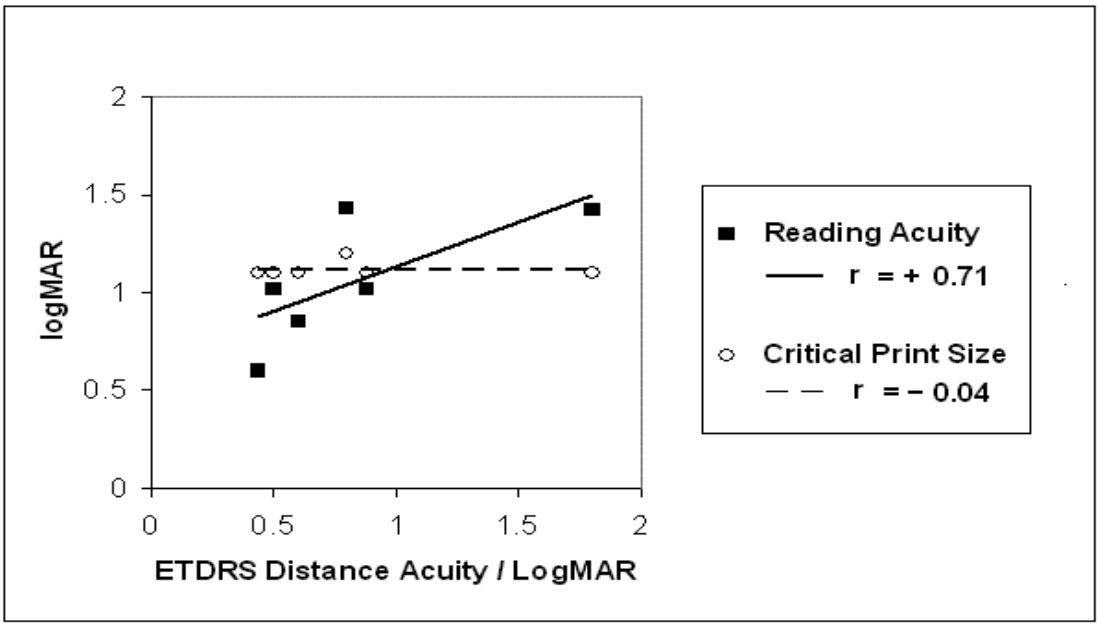
Saccadic assessment when reading revealed the MT360 cohort displayed a greater number of forward saccades than the age matched normal group ($P<0.05$) but less than the dry ($P>0.05$) and wet ($P<0.05$) AMD groups. There was no significant difference between the groups for the number of regressive saccades performed (Table 35).

Table 33 Outcome Measures MT360 Cohort: Pre-operative vs. Post-operative

Variable	Pre-operative	Post-operative
ETDRS Distance Acuity / logMAR	**0.70** (0.44 – 1.80)	**0.25** (0.10 – 0.60) **P = 0.09**
MN Read™ Reading Acuity / logMAR	**1.02** (0.60 – 1.43)	**0.52** (0.22 – 0.66) **P = 0.01**
MN Read™ Reading Speed / words per minute	**106** (52 – 150)	**158** (55 – 231) **P = 0.14**
MN Read™ Critical Print Size / logMAR	**1.10** (1.10 – 1.20)	**0.80** (0.50 – 1.10) **P = 0.05**
Pelli-Robson Contrast Sensitivity / logMAR	**0.75** (0.00 – 1.05)	**1.43** (1.20 – 1.65) **P = 0.02**

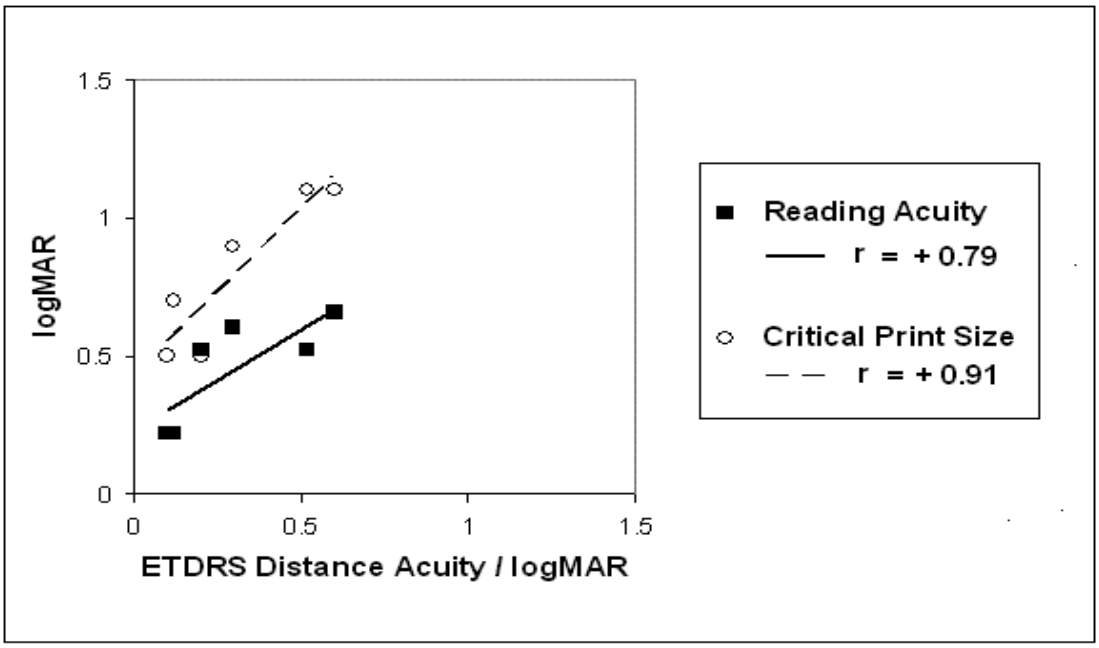
Values Represent Median (Range) and Significance Levels

Figure 4 Relationship Between Pre MT360 Distance Acuity + Reading Function



r Coefficient of Correlation

Figure 5 Relationship Between PostMT360 Distance Acuity + Reading Function



r – Coefficient of Correlation

Table 34 Outcome Measures: MT360 vs. Comparative Groups

Outcome	MT360	Normal	Dry AMD	Wet AMD
ETDRS Acuity / logMAR	**0.25** (0.10 – 0.60)	**0.02** (- 0.14 – 0.14) **P = 0.004**	**0.57** (0.44 – 1.10) **P = 0.038**	**0.67** (0.38 – 1.18) **P = 0.013**
MN Read™ Reading Acuity / logMAR	**0.52** (0.22 –0.66)	**0.10** (- 0.11 – 0.29) **P = 0.002**	**0.64** (0.52 – 1.15) **P = 0.038**	**0.72** (0.54 – 1.27) **P = 0.009**
MN Read™ Reading Speed / words per minute	**158** (55 – 231)	**151** (116 – 194) **P = 0.587**	**94** (44 – 173) **P = 0.030**	**51** (28-99) **P = 0.003**
MN Read™ Critical Print Size / logMAR	**0.80** (0.50 – 1.10)	**0.25** (0.10 – 0.40) **P = 0.001**	**0.95** (0.70 – 1.30) **P = 0.324**	**1.00** (0.80 – 1.30) **P = 0.101**
Pelli-Robson Contrast Sensitivity / logMAR	**1.43** (1.20 – 1.65)	**1.65** (1.50 – 1.70) **P = 0.042**	**1.38** (0.70 – 1.70) **P = 0.702**	**1.38** (0.90 – 1.65) **P = 0.585**

Values Represent Median (Range) and Significance Levels

Figure 6 Distance Acuity: MT360 vs. Comparative Groups

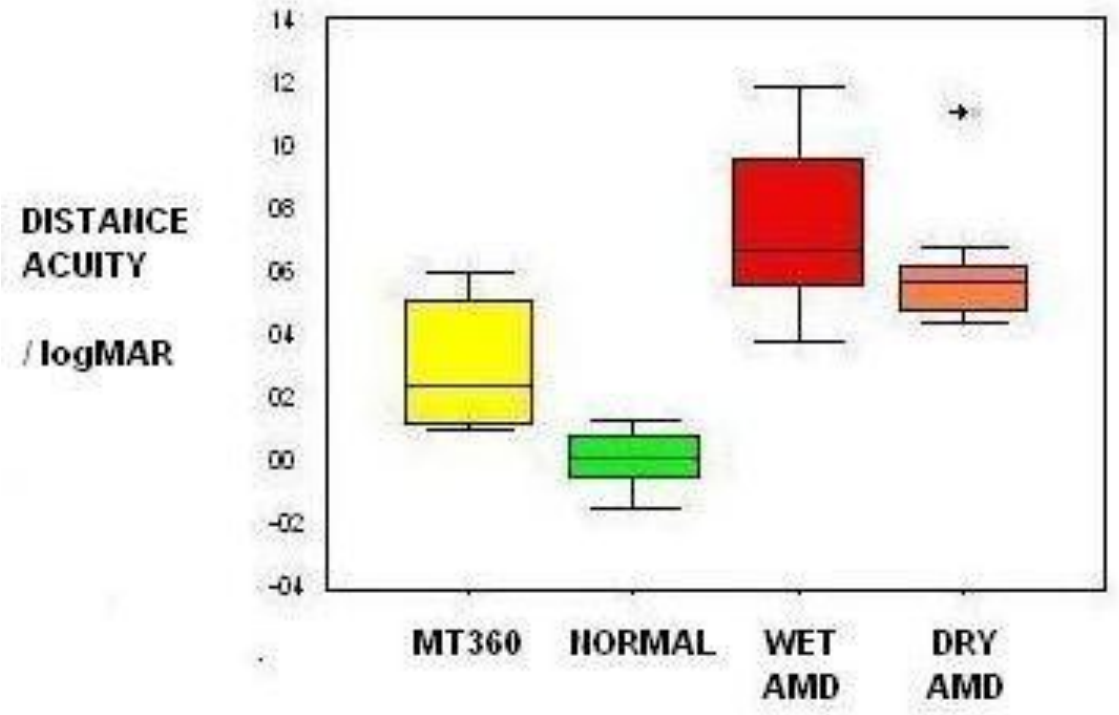


Figure 7 Reading Acuity: MT360 vs. Comparative Groups

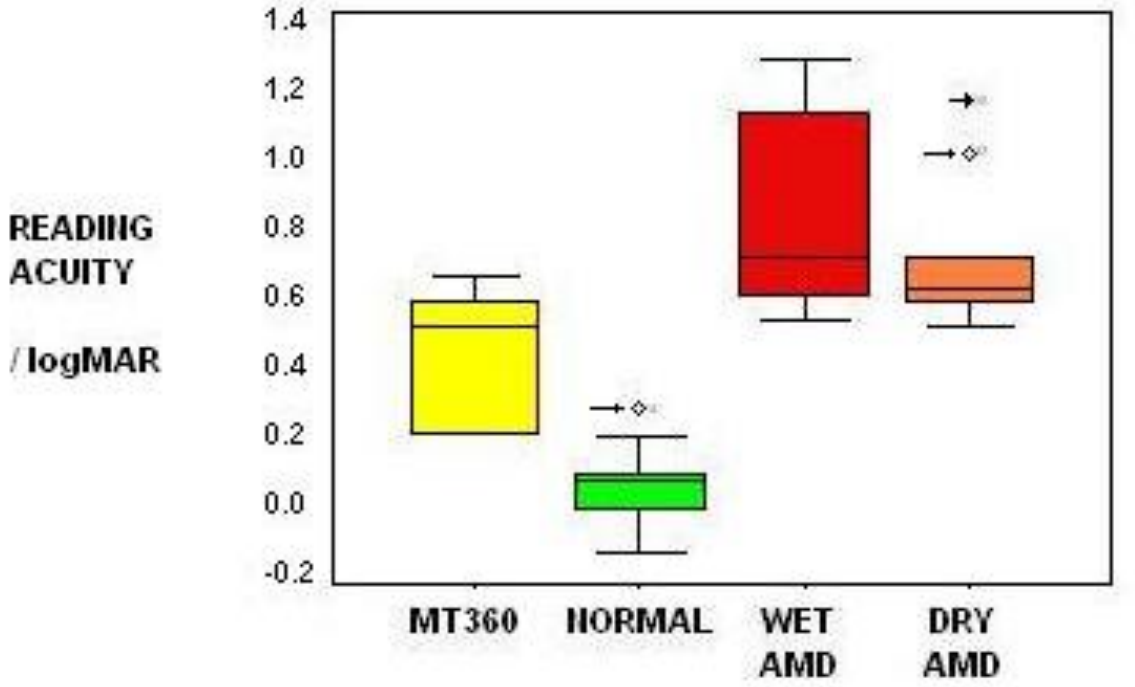


Figure 8 Reading Speed: MT360 vs. Comparative Groups

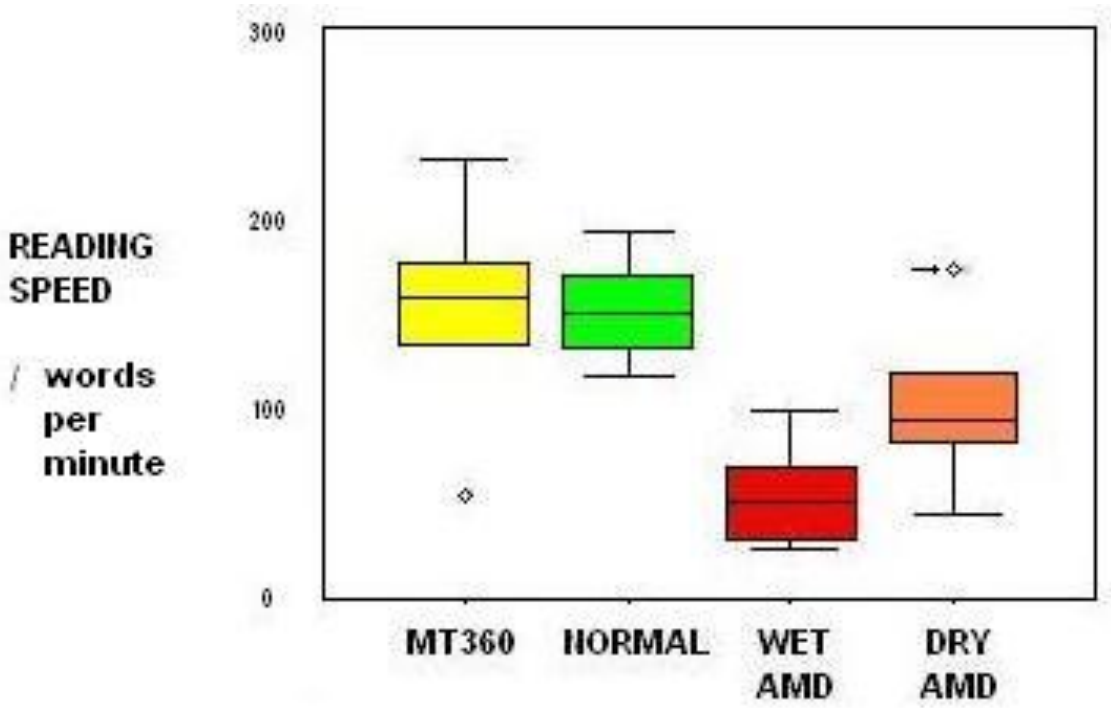


Table 35 Horizontal Saccade and Reading Task: MT360 vs. Comparative Groups

Horizontal Saccades	MT360	Normal	Dry AMD	Wet AMD
Number of Saccades	**2.44** (2.15 – 5.56)	**2.00** (1.25 – 1.45) **P = 0.009**	**1.84** (1.40 – 2.80) **P = 0.008**	**2.35** (1.30 – 3.89) **P = 0.385**
Latency / ms	**269** (200 – 291)	**225** (156 – 542) **P = 0.170**	**238** (159 – 304) **P = 0.071**	**209** (172 – 331) **P = 0.083**
Velocity / ms^{-1}	**364** (306 – 427)	**479** (207 –1904) **P = 0.050**	**459** (366 – 655) **P = 0.013**	**470** (225 – 631) **P = 0.023**
Forward Saccades	**13.5** (10.1 – 25.1)	**8.7** (7.1 – 10.4) **P = 0.002**	**17.3** (10.0 – 30.5) **P = 0.447**	**29.1** (17.0 – 50.2) **P = 0.002**
Regressive Saccades	**29.4** (20.3 – 42.7)	**31.5** (7.0 – 43.0) **P = 1.000**	**12.0** (5.0 – 43.0) **P = 0.127**	**33.0** (21.0 – 70.0) **P = 0.514**

Values Represent Median (Range) and Significance Levels

Figure 9 Horizontal Saccades: MT360 vs. Comparative Groups

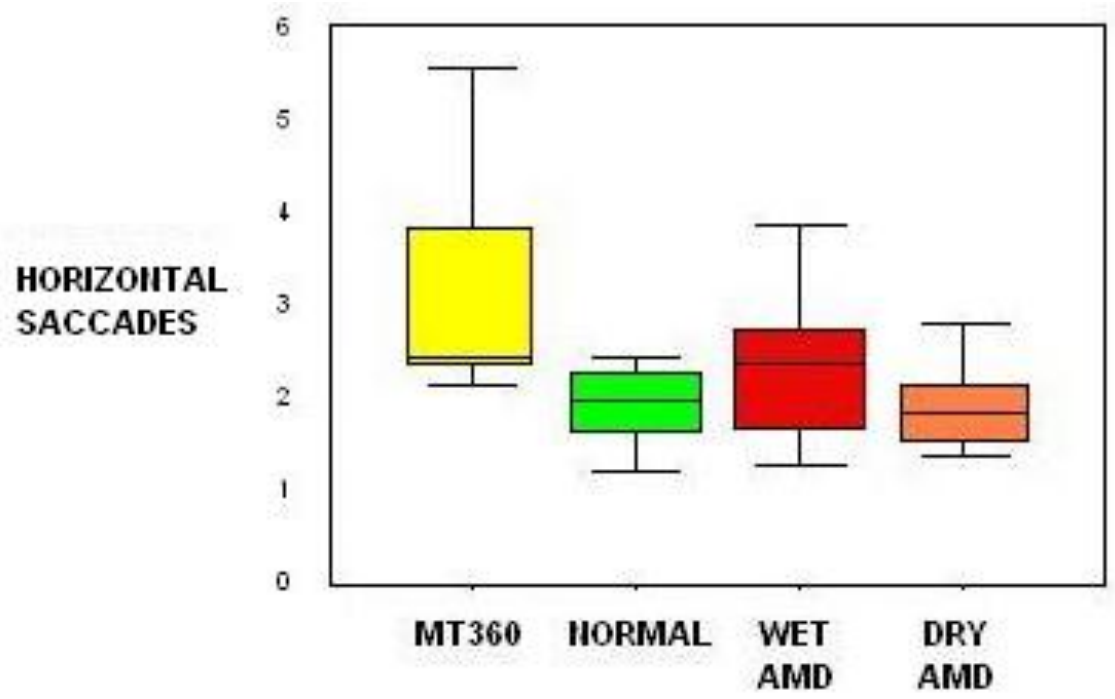


Figure 10 Horizontal Saccade Latency: MT360 vs. Comparative Groups

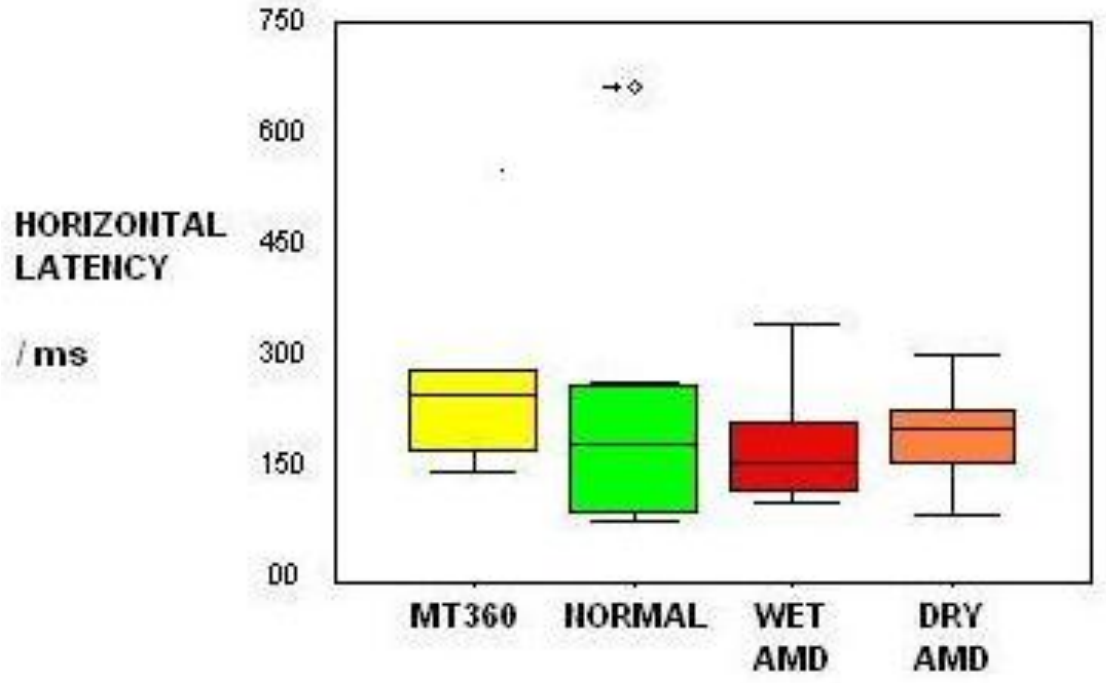
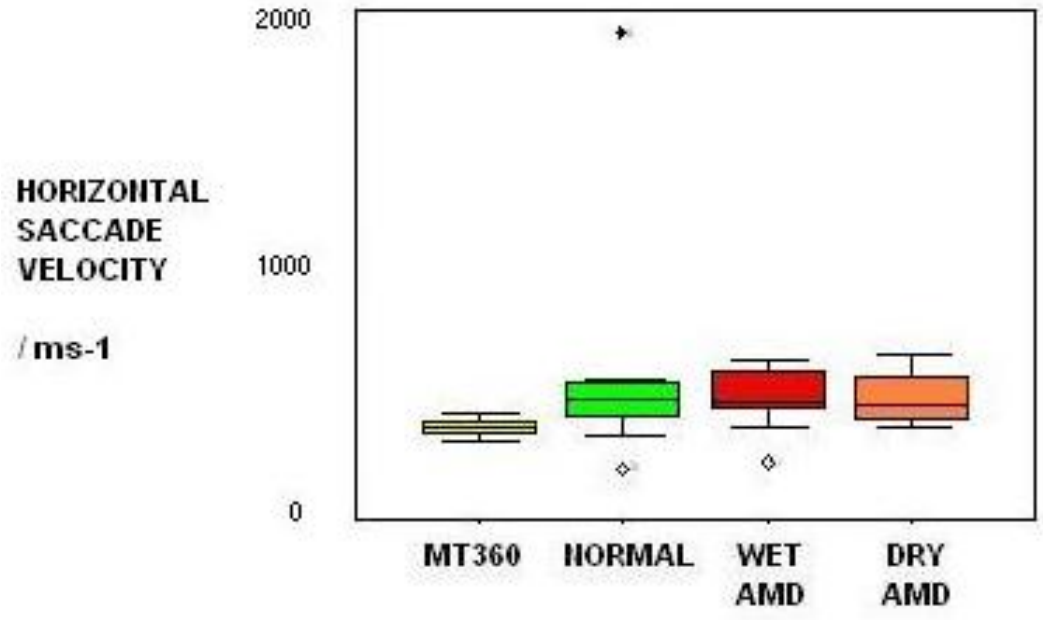


Figure 11 Horizontal Saccade Velocity: MT360 vs. Comparative Groups



Vertical Saccade Task

Assessment of vertical saccades demonstrated the MT360 cohort performed more vertical saccades ($P<0.05$) of increased latency ($P<0.05$) with a reduced saccade velocity ($P>0.05$) when compared to age matched normal subjects. A similar pattern of results was demonstrated between the MT360 cohort and the AMD groups although this failed to reach significance (Table 36).

Reading Speeds

Assessment of reading speeds revealed the MT360 cohort outcomes approximated toward the normal group and were greater than the AMD groups. The improvement following MT360 was found to be significantly different from normal group and the wet AMD group ($P<0.05$) but not the dry AMD group ($P>0.05$) (Table 37). Reading speeds as calculated from MN Read™ Reading Acuity Charts revealed a similar pattern and are shown for comparison in Table 37.

Eyetracker Assessment of Fixation

Analysis of fixation revealed the fixation stability global BCEA values for the MT360 cohort to be closer to the normal group than to the AMD groups. No significant difference ($P>0.05$) was found between the MT360 cohort and any comparative group. However analysis of global BCEA values revealed a single preferred retinal locus for the MT360 and normal age controls ($P<0.05$) with multiple loci for both AMD groups ($P<0.05$) (Table 38) (Figure 12).

Microperimetry Assessment of Retinal Sensitivity and Fixation

Microperimetry assessment of macular sensitivity demonstrated that for three (Patient 1, 3 and 4) of the six patients in the MT360 cohort, surgery restored near normal levels of foveal and para-foveal retinal sensitivity (Figure 13). Only Patient 6 demonstrated a significant residual absolute scotoma.

Microperimeter fixation analysis demonstrated stable fixation for all patients in the surgical cohort. The median percentage of time that the target was placed within 2º and 4º of the fovea over the 30 seconds of the test period was 90% (72 – 100) and

96% (86 – 100) respectively (Table 39). Pre and post MT360 BCEA values from the MP-1 are also shown for comparison.

Table 36 Vertical Saccades: MT360 vs. Comparative Groups

Vertical Saccades	MT360	Normal	Dry AMD	Wet AMD
Number of Saccades	**2.32** (1.95 – 3.00)	**1.76** (1.50 – 5.25) **P = 0.044**	**2.20** (1.37 – 4.94) **P = 0.129**	**2.09** (1.39 – 3.82) **P = 0.551**
Latency / ms	**249** (211 – 297)	**197** (163 – 335) **P = 0.039**	**217** (198 – 306) **P = 0.448**	**234** (165 – 333) **P = 0.129**
Velocity / ms^{-1}	**378** (289 – 459)	**446** (224 – 647) **P = 0.193**	**404** (216 – 499) **P = 0.914**	**378** (201 – 644) **P = 0.828**

Values Represent Median (Range) and Significance Levels

Table 37 Eyetracker Reading Speed: MT360 vs. Comparative Groups

Sentences	MT360	Normal	Dry AMD	Wet AMD
Eyetracker Sentences Reading Speed	**101** (57 – 104)	**139** (107 – 183) **P = 0.039**	**79** (41 – 108) **P = 0.114**	**39** (24 – 59) **P = 0.002**
MN Read™ Reading Speed	**158** (55 – 231)	**151** (116 – 194) **P = 0.587**	**94** (44 – 173) **P = 0.030**	**51** (28 – 99) **P = 0.003**

Values Represent Median (Range) and Significance Levels

Table 38 Eyetracker Assessment of Fixation and Number of Preferred Retinal Loci

Fixation Analysis	**MT360**	**Normal**	**Dry AMD**	**Wet AMD**
Global BCEA / min arc^2	**310** (49 – 1890)	**116** (50 – 3081) **P = 0.515**	**1426** (175 – 13078) **P = 0.065**	**651** (59 – 6465) **P = 0.329**
Preferred Retinal Loci	**1.0** (1.0 – 2.0)	**1.0** (1.0) **P = 0.197**	**2.0** (1.0 – 4.0) **P = 0.006**	**3.0** (2.0 – 7.0) **P = 0.001**

Values Represent Median (Range) and Significance Levels

BCEA – Bivariate Contour Ellipse Area

Figure 12 Number of Preferred Retinal Loci: MT360 vs. Comparative Groups

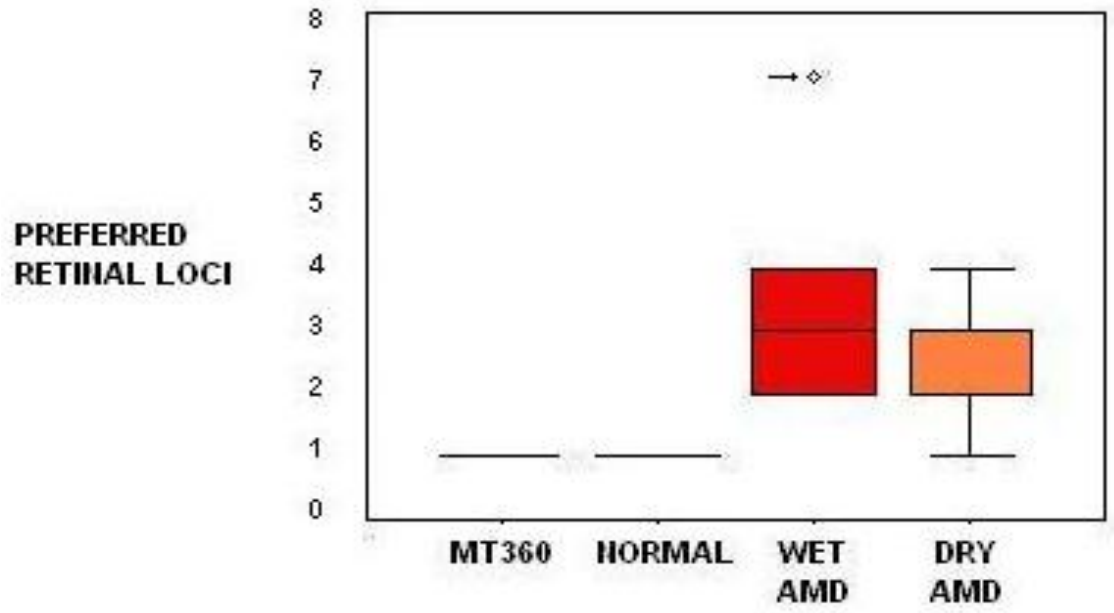


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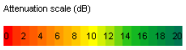
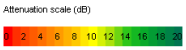
Figure 13 Nidek MP – 1 Assessment of Retinal Sensitivity: MT360 Cohort



Patient	Macular Sensitivity	Foveal Sensitivity
1		
2		
3		

Figure 13.1 Nidek MP – 1 Assessment of Retinal Sensitivity: MT360 Cohort



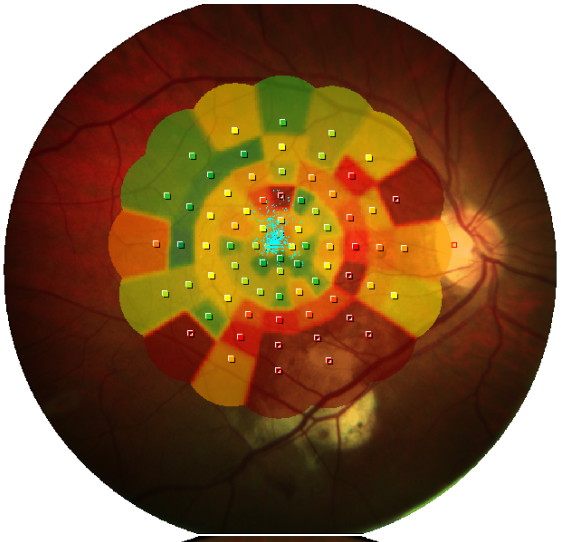
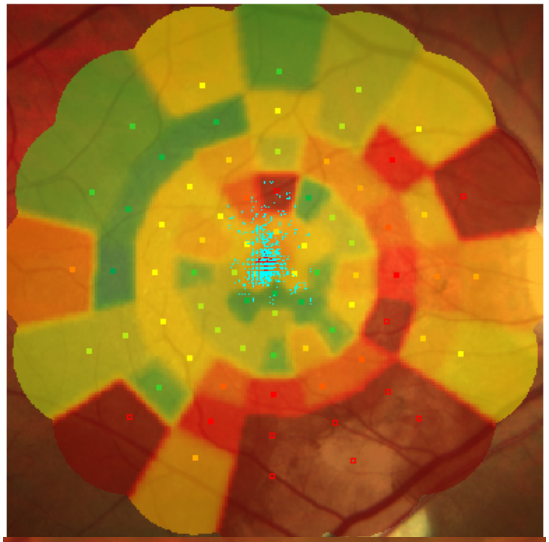
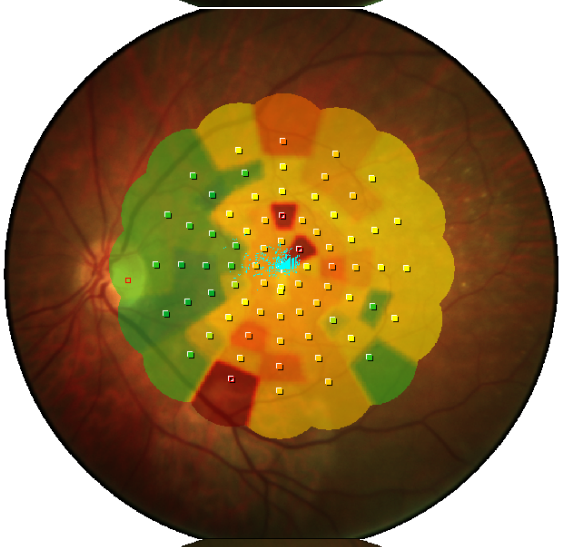
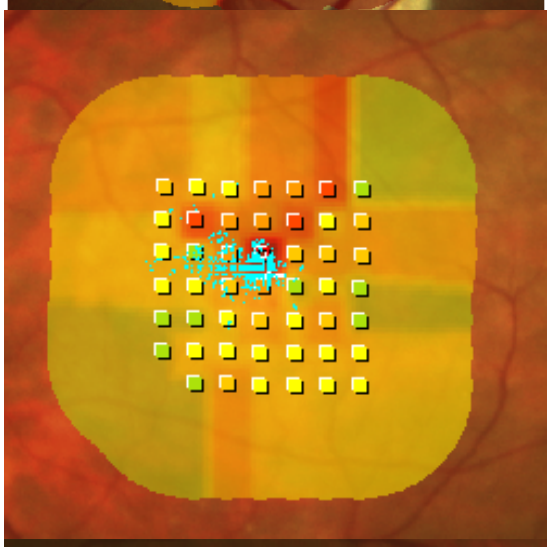
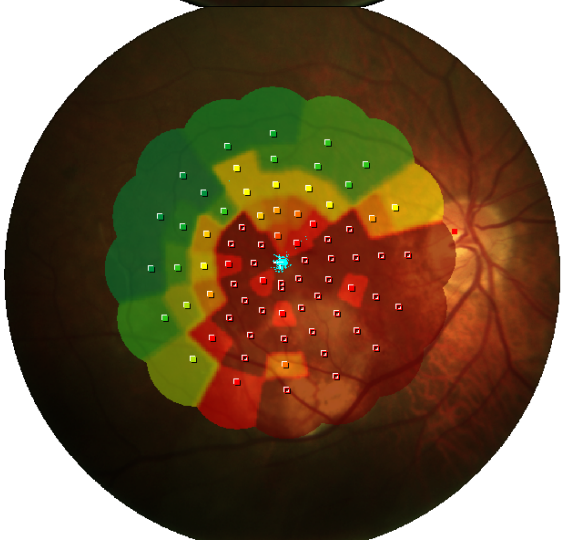
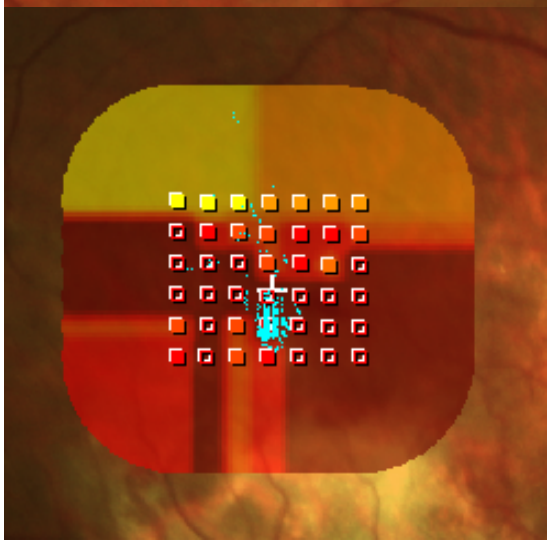
Patient	Macular Sensitivity	Foveal Sensitivity
4		
5		
6		

Table 39 Nidek MP - 1 Assessment of Fixation

Patient	Mean % Time Fixation within 2 ° of Target	Mean % Time Fixation within 4 ° of Target	Nidek MP-1 Classification of Fixation	Nidek MP-1 Bivariate Contour Ellipse Area (min arc^2)	
				PreOp	PostOp
1	99	99	High Quality Stable	2160	360
2	77	95	High Quality Stable	612	216
3	100	100	High Quality Stable	720	756
4	98	98	High Quality Stable	1512	1,656
5	95	97	High Quality Stable	2988	792
6	72	86	High Quality Stable	12,780	3096

High Quality Fixation = Greater than 50% of the preferred fixation points located within a 2º diameter circle centred on the fovea

Stable Fixation = Greater than 75% of fixation points located within a 2º diameter circle centred on the gravitational centre of all fixation points.

Discussion

MT360 differs from current treatments for severe AMD that aim to modify the disease process. Instead, translocation offers a unique rescue procedure for foveal photoreceptors in patients whose fellow eye has already lost central vision. Previous reports of translocation have reported this rescue in terms of acuity alone (Eckardt C, 1999, Pertile and Claes, 2002, Aisenbrey et al., 2002b). More recently several authors have examined the impact of MT360 on near visual function and reading ability (Lai JC, 2002, Mruthyunjaya et al., 2004, Fujikado et al., 2002a, Toth et al., 2004). While these studies provide good evidence for the recovery of foveal photoreceptor function by mid-peripheral retinal pigment epithelium (RPE), none have described the quality and extent of this recovery compared to normal visual function.

MT360 is an involved procedure encompassing both retinal and strabismus surgery. By observing the best outcome cases of MT360 in our surgical series this study has attempted to gauge the optimal potential of this complex surgery in restoring normal function. The study has documented not only the well recognised components of near visual function (reading acuity, contrast sensitivity reading speeds, critical print size) but also aspects of reading behaviour (number, duration and velocity of saccades), fixation characteristics (preferred retinal loci and stability of fixation), and retinal sensitivity (size, location and density of any post-operative scotoma) and compared these parameters to both untreated disease and normal function.

The results of the first 23 consecutive patients treated with MT360, including the surgical learning curve, illustrate a restoration of distance and reading acuity. Despite the overall distance acuity change failing to reach significance, the results compare favourably with previous investigations into MT360 (Eckardt C, 1999, Lai JC, 2002, Pertile and Claes, 2002, Mruthyunjaya et al., 2004, Fujikado et al., 2002a, Toth et al., 2004). The best outcome cases were recruited for eyetracker analysis of reading behaviour and microperimetry. At 14 months follow up the measured parameters of visual function all improved in this cohort. MT360 produced a significant improvement in reading acuity ($P<0.01$), critical print size ($P<0.05$) and contrast sensitivity

(P<0.02), however improvements in distance acuity and reading speed did not reach significance.

The latter results are likely a function of sample size however several studies of MT360 have reported a greater improvement in near over distance acuity (Eckardt C, 1999, Lai JC, 2002, Mruthyunjaya et al., 2004, Fujikado et al., 2002a). Other than sample size, the discrepancy in near over distance vision is difficult to explain and may reflect the complex interplay of location, density and size of a residual scotoma on reading function. A possible explanation is that MT360 results in a reduction in the foveal scotomatous area without an improvement in the overall retinal sensitivity, thus resulting in a greater improvement in near function. Additionally, alterations in micro-saccadic behaviour following counter-rotation surgery (discussed below) may have more of a deleterious effect on distance acuity than near reading ability.

The failure of reading speed (which is not limited by print size) to significantly improve reflects the fact that a reasonable speed may be attained pre-operatively (at a larger print size) and only marked improvements in this parameter will reach significance. In this respect, the critical print size (smallest print read with maximum speed), which significantly improved following MT360, better reflects improvements in reading ability.

Pre-operatively distance acuity was well correlated (r = + 0.71) with reading acuity as expected, however there was no such correlation (r = - 0.04) with critical print size (Figure 4). Patients suffer a profound central scotoma in their previously better second eye and it is predominately the loss of reading ability and not distance acuity that commonly instigates presentation. The poor reading stems from the scotoma forcing eccentric viewing (Timberlake et al., 1986), an unstable locus for fixation (Crossland et al., 2004a, Crossland et al., 2005a, Bellmann et al., 2004a, Culham et al., 1993) and compromised eye movement control (White and Bedell, 1990, McMahon et al., 1991, Whittaker et al., 1991). In the long term patients may develop adaptive mechanisms to partially overcome these problems, however a requirement of successful MT360 is a short duration between acute visual loss and surgery (Uppal et al., 2007). Thus, pre-operatively, while patients may still resolve smaller print sizes, the critical print size remains relatively poor and independent of distance

and reading acuity. The results again demonstrate that both distance and reading acuity are inadequate measures of reading abilities.

Post-operatively, distance acuity was well correlated with both the reading acuity (r = + 0.79) and critical print size (r = + 0.91) (P<0.001) (Figure 5). Surgery dramatically reduced or reversed the absolute central scotoma, recovering levels of macular sensitivity approaching normal (Figure 13) with stable central fixation (Tables 31 and 32; Figures. 12 and 13). MT360 closely approximates foveal anatomy to a normal physiological state and this is reflected in significant improvements in function (reading acuity, critical print size, contrast sensitivity) and hence reading ability. These results support the premise that restoration of the choriocapilliaris-Bruch's-RPE-photoreceptor axis rescues foveal function and this can be achieved with mid-peripheral RPE substituting for diseased sub-foveal RPE.

To gauge the extent of the recovery detailed above, outcome measures (distance acuity, reading acuity, reading speed, critical print size and contrast sensitivity), eyetracker assessment of reading behaviour, and microperimetry were examined, comparing, where possible, the MT360 cohort to age matched normal subjects and patients with untreated wet and dry AMD. The comparison revealed that the recovery following MT360 was greater than either of the untreated AMD groups and less than the age matched normal group (Table 27, Figures 6,7 and 8). The results are consistent with an improvement in the absolute central scotoma with restoration of the macular threshold function and resolution of the size and or density of the pre-operative scotoma. As microperimetry was not available pre-operatively, changes in retinal threshold function cannot be quantified directly, although all patients in the surgical cohort presented with large sub-foveal haemorrhages and a recent loss of reading ability. Although microperimetry revealed that surgery re-approximates macular sensitivity towards normal levels, a relative scotoma persists (Figure 13). This is likely to account for reduced reading ability and partly reflect the failure of the improvement in contrast sensitivity and critical print size to reach significance (Silverstone et al., 2000). This would also explain the failure of the surgical cohort to match outcomes compared to normal subjects except for reading speed. As discussed above, although critical print size better reflects reading ability, it is of interest to note that patients post translocation/counter-rotation were able to recover

sufficient function to achieve a normal level of maximum reading speed (Table 27, Figure 8)

In addition to near normal levels of macular sensitivity, Patient 1, 3 and 4 also displayed the best three post-operative outcomes (Table 24). Of note Patient 1 had the best pre-operative vision while Patient 3 and 4 the worst. This suggests that while good pre-operative vision will result in good post-operative outcomes, the limiting factor for this rescue procedure is likely to be the number and quality of the photoreceptor pool prior to surgery and not necessarily acuity. It has previously been demonstrated that a significant photoreceptor loss occurs in patients with AMD beginning in the para-fovea (Curcio et al., 1996). Electrophysiological studies have also demonstrated that wider retinal and RPE areas are involved in AMD than are evident from fundoscopy (Luke et al., 2001). Furthermore, ultra structural studies have found a partial loss of photoreceptor outer segments following translocation, thought to be torn away during separation of the retina from the RPE (Machemer R, 1993a). Therefore, considering that surgical recovery is undertaken on the background of an ageing and diseased retina, and the physical processes involved in MT360 will result in a further loss of photoreceptors, recovery of truly normal function is less likely.

Fixation analysis established that translocation produces a single stable central retinal locus – the new foveal location, whereas the AMD groups demonstrated multiple loci. The eyetracker demonstrated that BCEA values for the surgical cohort were far closer to age matched normal subjects than either AMD group (Table 31 and Figure 12) and microperimetry similarly confirmed high quality, central fixation (Table 32 and Figure 13).

Eyetracker assessment of saccadic behaviour revealed that translocation results in a greater number of horizontal saccades ($P<0.05$ - except wet AMD) of reduced velocity ($P<0.05$) and increased latency ($P>0.05$) as compared to the other groups (Figures 9,10 and 11). The MT360 also displayed a greater number of forward saccades than age matched normal subjects ($P<0.05$) but fewer than the dry AMD ($P>0.05$) and wet ($P<0.05$) groups with no difference in the number of regressive saccades between groups. Examination of vertical saccades, which are implicated in

finding a new line when reading, revealed a similar pattern (Table 29). In the presence of high quality stable fixation, these results reflect the direct effects of counter- rotation surgery upon gaze control during reading rather than deficits in macular function. Counter-rotation surgery (which involves transposition of 2 – 4 recti muscles) is necessary to correct post translocation torsion to less than 10º (when it is considered that symptoms of diplopia and tilt are negligible). Despite the suboptimal oculomotor dynamics highlighted above, the MT360 cohort of patients are able to adapt and achieve near normal reading ability.

MT360 is a complex two-stage procedure after which patients have a reduced peripheral field (secondary to 360º retinectomy performed during retinal translocation) and a degree of residual torsion (secondary to counter-rotation surgery). This study has additionally demonstrated, with infrared eyetracking, that when performing reading tasks following surgery patients perform an increased number of saccades of slower velocity and greater latency. Eyetracking and microperimetry also confirm the restoration of a single stable foveal locus of fixation. Furthermore microperimetry reveals post-operative macular sensitivity approaches normal levels leaving a relative central scotoma.

The purpose of this study was to gauge the optimal potential of MT360 in rescuing function and describing the quality and extent of the recovery. Despite the profound anatomical disruption that MT360 induces, the results indicate that retinal surgery approximates normal macular threshold levels, and the limitations placed on oculomotor control appear not to adversely affect reading ability. Although the findings are clearly limited by the small sample sizes involved, the present study nevertheless shows that MT360 can successfully rescue photoreceptors with a significant improvement over untreated disease for distance acuity, near acuity and reading speed.

V RPE Transplantation: Age Related Macular Degeneration

Introduction

AMD is characterized by the growth of choroidal new vessels (CNV) under the central macula, which may lead to acute visual loss by hemorrhage or serous detachments of the retinal pigment epithelium (RPE) and/or neurosensory retina. The acute visual loss associated with CNV is widely believed to be the result of initially sub-retinal pathology, which impairs function of the overlying neurosensory retina (Hageman et al., 2001, Ambati et al., 2003). Over time, the chronic impairment of the neurosensory retina most likely leads to irreversible photoreceptor apoptosis (Dunaief et al., 2002, Piccolino et al., 2005). The aim of AMD surgery is therefore to restore contact between the macula and healthy sub-retinal tissue (RPE and choroid), before significant photoreceptor loss occurs. The concept of reversing photoreceptor loss of function has already been shown with macular translocation.

It has previously been shown and recently confirmed that removal of CNV alone does not lead to an improvement over nonintervention in the management of acute AMD (Thomas et al., 1994, Hawkins et al., 2004b). This most likely occurs because the sub-foveal RPE and choriocapilliaris are damaged as a result of CNV growth and/or its subsequent removal, and are therefore no longer able to support foveal function (Gass, 1994). This concept also led Machemer and Steinhorst (1993) (Machemer R, 1993b) to develop macular translocation surgery as a means of restoring foveal contact to healthy RPE, which has been further refined with more effective surgical techniques to reduce complications (Eckardt C, 1999, Mruthyunjaya et al., 2004). Visual improvement after macular translocation is strong proof of the principle that foveal dysfunction in AMD can be reversed, at least in the early stages, by re-apposition of the fovea to healthy RPE choroid of non-foveal origin.

A subsequent study proposed by Stanga et al (2002) (Stanga et al., 2002) explored surgery to the choroid as an alternative to macular rotation. This has the added advantage of avoiding image cyclotorsion, which would otherwise cause torsional

diplopia in patients with good vision in the fellow eye. The surgery involved cutting a 2- to 3-mm triangular wedge of sub-macular choroid with attached RPE and repositioning it under the fovea after CNV removal in 1 continuous procedure. The technique was not, however, successful in maintaining vision at the 5 year follow-up, possibly because the neuroretina was already irreversibly damaged, either before surgery or by the procedure itself (MacLaren et al., 2005). van Meurs and Van den Biesen (2003) (van Meurs and Van Den Biesen, 2003) proposed a similar approach, but one using full-thickness RPE choroid grafts harvested from the equatorial retina. Equatorial RPE is abundant, contributes little to functional vision, and is relatively unaffected by AMD. As such it is a good donor site for this technique. Significantly, they noted that these grafts could become reperfused when placed into the sub-foveal space, a critical prerequisite for supporting retinal function. This had a further benefit in that grafts could be larger and harvested with considerably less sub-foveal instrument manipulation (van Meurs and Van Den Biesen, 2003). The improvement in vision noted in some patients provided strong evidence that foveal photoreceptors could be supported by RPE harvested from the equator, despite the reduced density and flatter morphology of the latter compared to RPE at the fovea (Gao and Hollyfield, 1992).

This study describes a prospective interventional cohort study to assess the surgical, anatomic, and functional effectiveness of peripheral RPE choroid transplantation in patients with severe vision loss due to acute neovascular AMD. The study also sought to gain a better understanding of surgery to the choroid, as this is a relatively unexplored procedure in ophthalmology.

Methods

Patients

A prospective series of 12 consecutive patients (2 female, 10 male) with a mean age of 80 (73 – 91) years and CNV secondary to AMD underwent autologous RPE transplantation with silicone oil tamponade and removal of oil at Moorfields Eye Hospital, London (Table 40). The study was conducted with full ethical approval as outlined in the General Methods.

Inclusion Criterion and Exclusion Criterion

Twelve patients were invited to take part in the trial with informed consent if they met all the following criteria:

Inclusion Criteria

- Acute sub-foveal neovascular AMD with or without sub-retinal hemorrhage.
- Ineligibility for conventional treatments, such as photodynamic therapy, which was not available in the UK for occult lesions during the course of this study and is contraindicated in the presence of an RPE rip. The pre-operative CNV classification for each patient is detailed in Table 40.
- Vision loss of at least 3 lines over 3 months before study entry.
- Clinical signs of CNV progression, as evidenced by increased sub-retinal hemorrhage or fluid, associated with the decline in vision.
- No more than 6 months delay between vision loss and surgery.

Exclusion Criteria

- Unfit for general anaesthetic
- Unwillingness to undertake follow-up
- Other ophthalmic pathology precluding complex vitreoretinal surgery
- Inability to give informed consent

Pre and Post-operative Assessments

Pre-operative examinations were performed as outlined in the General Methods. In addition patients underwent a subjective refraction with Early Treatment Diabetic Retinopathy Study visual acuity, and cataract grading using the Lens Opacities Classification System III (Tyrrell and Wolfe, 1993). All patients underwent pre-operative assessment at 6 weeks to check for CNV recurrence and graft revascularization and all tests were repeated at 6 months, which was the defined end point of the cohort study. Selected patients also underwent additional assessment of retinal sensitivity and fixation pattern after 6 months using a Nidek MP1 microperimeter (Nidek Co.,Ltd., Tokyo, Japan) as described in the General Methods and Appendix 2

Surgical Technique

Surgery was performed by 2 surgeons (GWA, LDC) at the study centre and closely followed the technique described elsewhere by van Meurs and Van den Biesen.14 Briefly, this involved a pars plana vitrectomy followed by CNV excision through a superonasal (left eye) or superotemporal (right eye) macular retinotomy. Haemostasis of the CNV bed was achieved by elevating the infusion bottle, and any surplus blood was aspirated through the retinotomy. Diode laser was then applied in a 2- to 3-mm square to the superior equatorial retina, which was subsequently cut out as a full-thickness RPE choroid graft after peeling off the overlying neurosensory retina. The graft was gripped on the choroidal surface with a customized aspirating cannula (Figure 14) designed by van Meurs (DORC Surgical Instruments, Zuidland, The Netherlands), and then slid through the original retinotomy into the sub-foveal space. A 5-ml syringe attached to the end of the cannula was used to create a sufficiently strong vacuum aspiration force, and gentle reflux released the graft once in position. A 3- to 4-mm diameter perfluorocarbon bubble was used to hold the graft during 2 to 4 further manipulations to ensure correct unfolding and a sub-foveal position. Retinopexy was not applied to the macular retinotomy and the eye was filled with silicone oil. Three months later, patients underwent phacoemulsification cataract surgery combined with silicone oil removal, followed by further procedures as required for any complications.

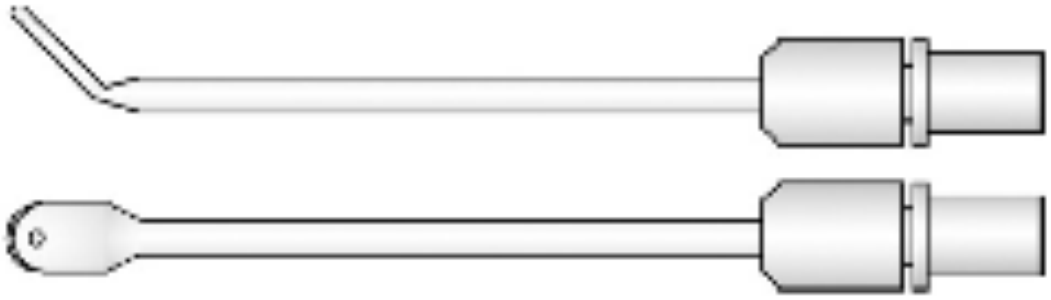
Electron Microscopy

For field emission scanning electron microscopy, a full thickness slice of RPE choroid was fixed and stored in a mixture comprising 3% glutaraldehyde and 1% paraformaldehyde in 0.08 mol sodium cacodylate buffer pH 7.4 at 4° C. It was then rinsed in 0.1 ml cacodylate buffer pH 7.4, fixed with 1% osmium tetroxide for 2 hours, dehydrated with 100% ethanol, critical point dried in liquid carbon dioxide, mounted, and coated with gold-palladium using a K550 sputter coater (Emitech, Ashford, United Kingdom). Secondary electron images of coated specimens were recorded digitally using an S4800 field emission scanning electron microscope (Hitachi, Maidenhead, United Kingdom) operating at 15 kV, as previously described (Erlandsen et al., 2004).

Gene Transfer

For gene transfer, a construct incorporating a cytomegalovirus promoter driving an enhanced green fluorescent protein (eGFP) reporter gene was incorporated into a self-inactivating third- generation human immunodeficiency virus vector, produced by a triple plasmid transient transfection system and pseudotyped with the VSV-G glycoprotein envelope as previously described (Dull et al., 1998). The virus was p24 titered using a Beckman Coulter (Fullerton, CA) kit following manufacturer instructions. For ex-vivo gene transfer studies, slices of rat RPE choroid were initially used to optimize culture conditions, virus concentrations, and exposure times. Surplus human RPE choroid tissue subsequently became available from 3 patients in the study group. The specimens were divided and co-cultured at 37° C in 5% CO2 within a 20-μl viral suspension containing 1.5 x 10^9 transducing units per milliliter in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY) for 20 minutes, 40 minutes, or overnight. After exposure to virus, specimens were washed three times in DMEM with Glutamax (L-analyl-L-glutamine, Gibco), 1:100 antibiotic-antimycotic solution (Gibco) and 3% fetal calf serum. The same solution was used for subsequent tissue culture at 37° C, which was examined daily for fluorescence and changed every 48 hours. Control specimens that had not been immersed in virus were co-cultured in contact with transduced tissue. After 1 week in culture, specimens were fixed in 4% paraformaldehyde, freeze embedded, and cut at 12 μm on a cryostat (Leica, Wetzlar, Germany). Sections were washed, counterstained with Hoechst 33342, and mounted.

Figure 14 The spatula designed by van Meurs and used in this study for holding and inserting the retinal pigment epithelium choroid graft into the sub-retinal space. The hole at the tip of the spatula is connected via the lumen to a fluid-filled syringe and tube fitted to the base connector. The graft is held in position by suction aspiration during surgery. Length, 60 mm.



Results

Presenting Features and Surgical Outcome

Patient demographics, duration of symptoms, and pre-operative status of the CNV and retina, and the pre-operative visual status are shown in Tables 40 and 41. Patient 2 had a wholly classic CNV, but it had become too large for photodynamic therapy after 2 previous unsuccessful treatments. All other patients had a serous or hemorrhagic pigment epithelial detachment or RPE rip. Three patients had pre-operative cystoid macular edema, which had re- solved at the final follow-up OCT scan. Retinal angiomatous proliferation was also present in 3 cases and, in 1 case (Patient 2), created an additional retinal break at the time of CNV excision. All cataracts were graded as mild by the Lens Opacities Classification System III assessment.16

The surgical procedure was technically successful in all 12 patients with regard to CNV excision and RPE grafting (Figure 15), taking 1 to 2 hours to perform. Dissection of the RPE choroid graft and mobilization on the spatula was relatively straightforward and was achieved without significant intra-operative hemorrhage. In 1 patient, the RPE choroid graft required trimming and a relatively large strip was removed en bloc from the eye. Electron microscopy of this specimen showed loss of sheets of RPE cells from the graft in some areas (Figure 16A), but the exposed underlying Bruch's membrane invariably remained intact (Figure 16B). The morphology of RPE cells was generally good, but occasional areas of single cell loss or loss of the RPE apex could be seen (Figure 16C).

Complications

Post-operative complications and visual outcomes are listed in Tables 42 and 43 and examples are shown in Figure 17. Patient 10 developed a large dome-shaped hemorrhage deep to the graft on the first post-operative day. This graft became surrounded by fibrous tissue and is the presumed appearance of complete graft failure (Figure 15E). Three other patients (6, 7, and 11) developed hemorrhages over

the graft within the first week of surgery. Graft-occluding hemorrhage occurring within the first week was described as a surgical complication because it would most likely be related to the procedure and might impede graft revascularization. A late hemorrhage would more likely be related to CNV recurrence as part of the ongoing AMD disease process. Patient 8 developed a hemorrhage at 6 weeks that, although extensive, did not track under the graft (Figure 17D) and eventually cleared to yield a reasonable visual outcome. No CNV recurrence was detected angiographically in any of the grafted patients. Retinal detachment occurred in 5 patients after combined cataract surgery with removal of silicone oil at 3 months and in all cases was associated with proliferative vitreoretinopathy (PVR). In 4 cases, silicone oil was reinserted and remained in the eye at most recent follow-up. Presumed causative retinal breaks were found at the edge of the donor site in Patient 1, at the hole created by removal of a CNV–retinal anastomosis in Patient 2, and from the CNV retinotomy sites in Patients 9 and 12 (Figure 17A).

Table 40 Patient Demographics, Duration of Symptoms, and Pre-operative Status of the CNV and Retina

Patient	Sex	Age	Laterality	Duration of Visual Loss / Weeks	Pathology	RPE Status	Neuroretina Status	PDT	Fixation	LOCS III Grading
1	F	76	OS	22	Occult	PED	CME + RAP	---	Good Central	N2C2
2	M	73	OD	14	Classic	---	CRA	2	Good Central	N2C2L1
3	F	76	OS	12	Occult	PED	RAP	---	Good Central	N2C2
4	M	75	OS	10	Minimally Classic	PED	H	---	Good Central	N1
5	M	75	OS	24	Occult	PED	H	---	Good Central	N1C1
6	M	86	OS	20	Occult	RIP	H		Good Central	N2C2
7	M	85	OD	24	SRH	PED	H	---	Good Central	N2
8	M	91	OD	04	SRH	HPED	H	---	Good Central	IOL

9	M	85	OD	16	Occult	RIP	CME	1	Good Central	N1C1L1
10	F	82	OD	12	Occult	RIP	H	---	Good Central	N1C1S1
11	M	78	OD	02	Occult	HPED	CME	---	Good Central	N1C1
12	F	77	OS	20	SRH	HPED	H	---	Good Central	C1

CME Cystoid Macular Oedema
CNV Choroidal New Vessels
CRA Chorioretinal Anastomosis from CNV
H Healthy Retina on Optical Coherence Tomography and Fundus Fluorescein Angiography
PED Pigment epithelial Detachment
HPED Haemorrhagic Pigment Epithelial Detachment
IOL Intraocular Lens
LOCS Lens Opacity Classification System;
PDT Photodynamic Therapy
RAP Retinal Angiomatous Proliferation
RPE Retinal Pigment Epithelium

The duration of symptoms represents as closely as possible the number of weeks over which the vision had fallen by 3 lines to the pre-operative level.

Table 41 Pre-operative Visual Status

Patient	Best Corrected Vision				Pelli-Robson Contrast Sensitivity	
	Distance		Near			
	Operated Eye	Fellow Eye	Operated Eye	Fellow Eye	Operated Eye	Fellow Eye
1	1.20	0.20	1.20	0.30	0.60	1.50
2	0.68	1.54	0.70	1.60	0.75	0.00
3	0.40	0.80	0.70	1.00	1.05	1.20
4	0.90	0.10	0.70	0.40	0.60	1.65
5	0.40	0.38	1.40	0.90	1.35	0.15
6	1.00	1.32	1.10	1.60	0.75	0.00
7	0.90	0.76	0.70	1.35	0.90	0.75
8	1.00	1.40	1.10	1.60	1.05	0.90
9	0.62	1.12	0.70	1.35	0.15	0.15
10	0.40	1.26	0.60	1.60	1.20	0.60
11	1.10	0.14	1.40	0.40	0.60	1.20
12	1.20	0.88	1.20	1.35	0.60	1.15

Visual acuities and contrast sensitivity are calculated as outlined in Appendix 1.

Table 42 Surgical Complications

Patient	Complications	PVR	Number of Operations*
1	RD	Yes	4+
2	RD	Yes	5+
3	ITU	No	2
4	None	No	2
5	None	No	2
6	Haem + RD	Yes	2
7	Hem	No	2
8	None	No	2
9	RD	Yes	3+
10	Hem	No	2
11	Hem	Yes	2
12	RD	Yes	3+

Haem Hemorrhage Around Graft within First Week
ITU Admitted to Intensive Therapy with a Presumed Cardiac Event
PVR Proliferative Vitreoretinopathy
RD Retinal Detachment
* Silicone Oil to be Removed at a Later Date.

Table 43 Post-operative Visual Status

Patient	Best Corrected Vision				Pelli-Robson Contrast Sensitivity	
	Distance		Near			
	Operated Eye	Change	Operated Eye	Change	Operated Eye	Change
1	1.30	-0.10	1.60	-0.40	0.00	-0.60
2	1.60	-0.92	1.20	-0.50	0.60	-0.15
3	1.60	-1.20	2.10	-1.40	0.00	-1.05
4	0.30	+0.60	0.50	+0.20	0.60	0.00
5	0.50	-0.10	1.30	+0.10	0.90	+0.45
6	1.40	-0.40	2.50	-1.40	0.00	-0.75
7	1.10	-0.20	1.30	-0.60	0.75	-0.15
8	0.60	+0.40	0.70	+0.40	NA	NA
9	1.00	-0.38	1.20	-0.50	NA	NA
10	1.60	-1.20	1.60	-1.00	0.90	-0.30
11	1.47	-0.37	1.40	0.00	NA	NA
12	1.47	-0.27	1.40	-0.20	NA	NA

Visual acuities and contrast sensitivity are calculated as outlined in Appendix 1.
NA Data Not Available

Figure 15 Fundal photographs and optical coherence tomography (OCT) images showing projection and sectional views of the retina, on the day before surgery (pre-op) and at 6 months (post-op), in 5 representative patients. Corresponding best-corrected visual acuities at subjective refraction are shown at the top right of each photograph, and OCT images represent vertical scans moving superiorly through the fovea, with the inferior retina represented to the left. A–C, Patients 4, 5, and 8 did well after autologous transplantation. D, Patient 7 had a successful graft with regard to revascularization but no gain in vision. E, Patient 10 had a primary failure of graft revascularization, presumed due to development of a large hemorrhage deep to the graft immediately post-operatively.

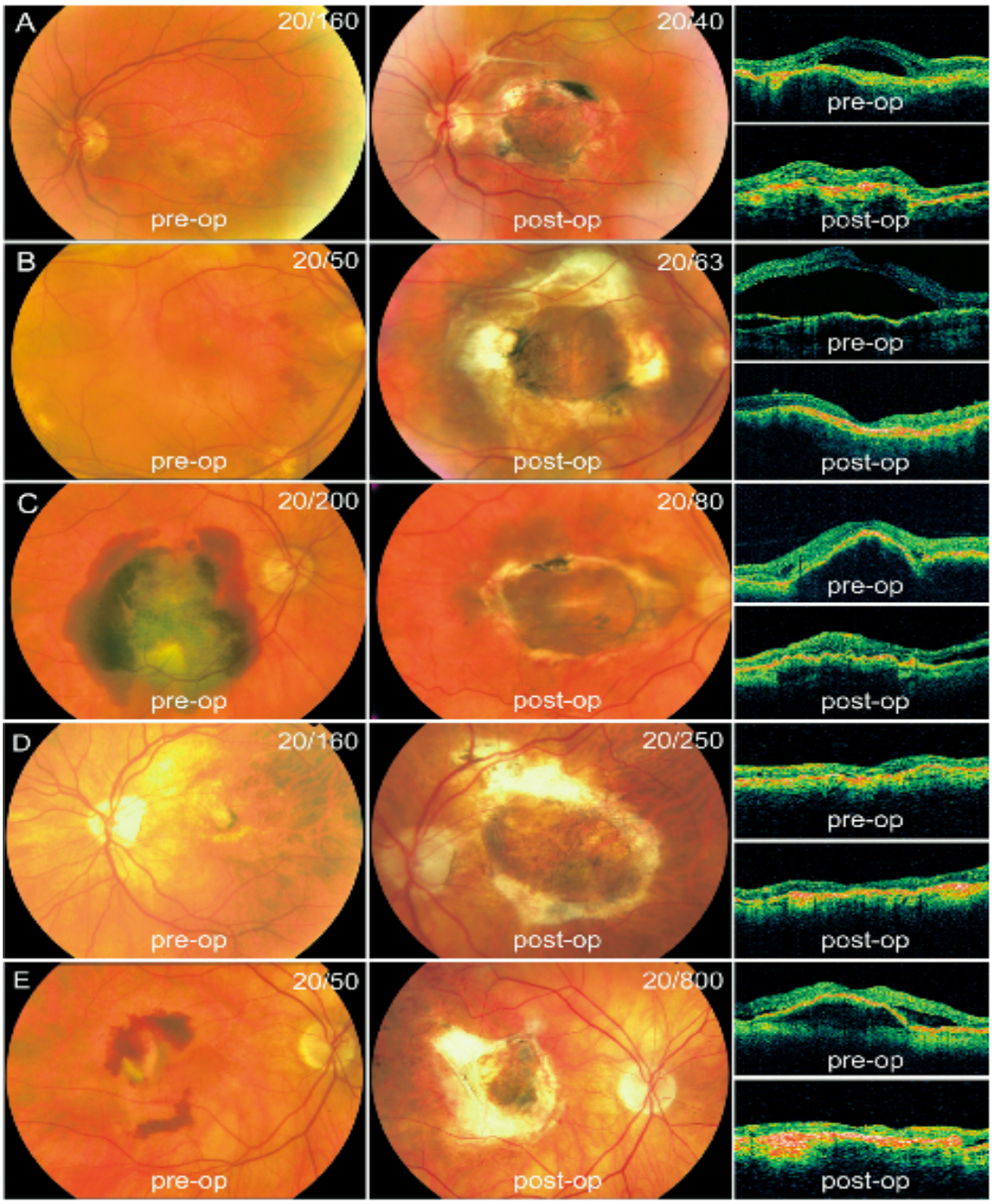


Figure 16 Field emission scanning electron microscope images showing a segment of a retinal pigment epithelium (RPE) choroid graft taken just after harvesting from the superior retina. Scale bars are shown beneath each image. **A**, Low-power view shows an area of denuded RPE (arrow) possibly caused by surgical trauma; boxed areas represent high-power views in B and C. **B**, Bruch's membrane underlying regions devoid of RPE cells. Note that the integrity of this layer is maintained despite focal RPE cell loss. C, Flat hexagonal structure characteristic of RPE cells in the equatorial region. Note the varying morphology of cells in this area, which reveals a healthy RPE cell with microvilli (top left arrow), a completely avulsed RPE cell with exposed underlying Bruch's membrane (middle arrow) and a partly de-roofed RPE cell exposing presumed phagocytosed outer segment discs (bottom right arrow)

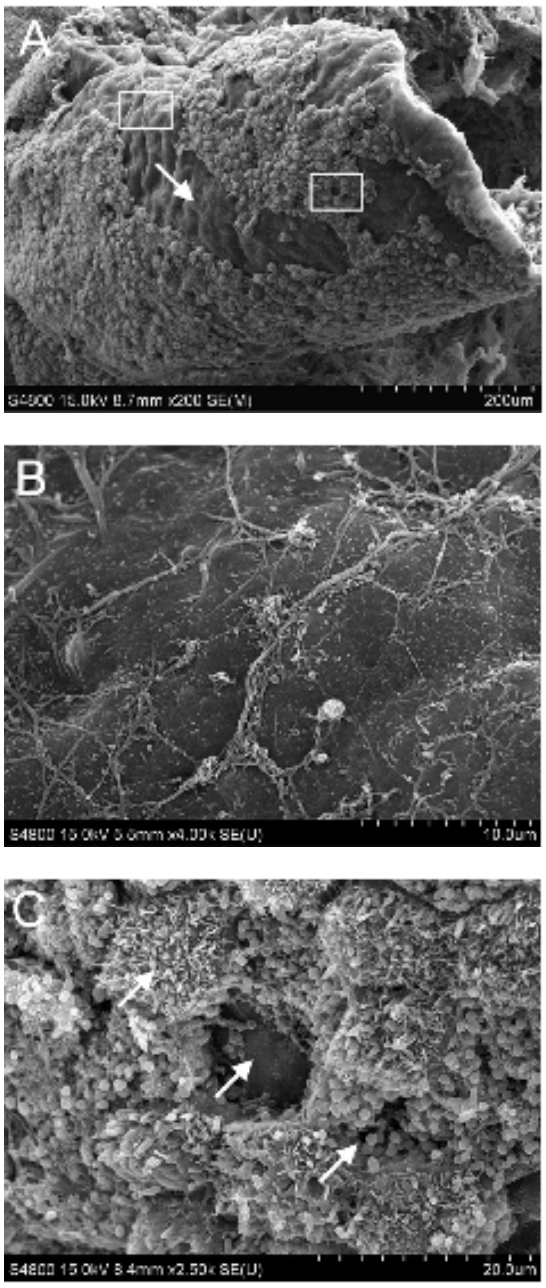
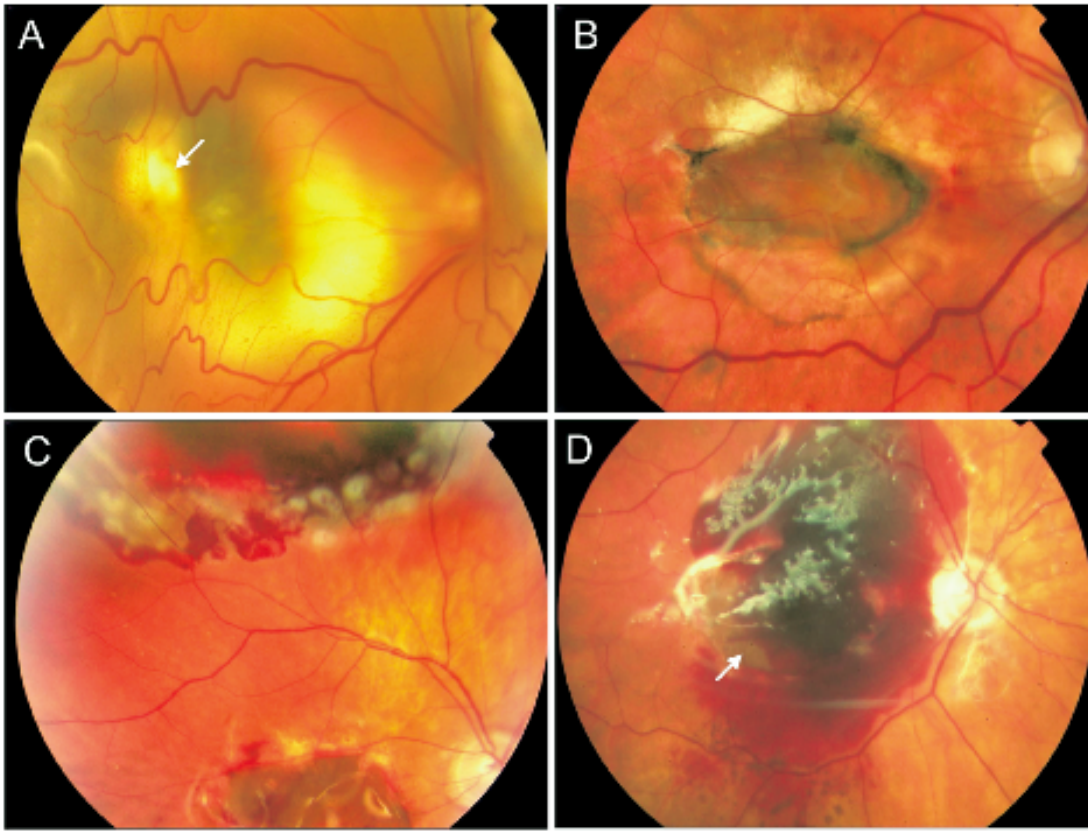


Figure 17 Complications arising from surgery. A, Retinal detachment due to proliferative vitreoretinopathy (PVR) (patient 9). Note that the graft has remained attached to the posterior sclera but the retinotomy site is adjacent to a PVR membrane and has reopened (arrow). B, Proliferative vitreoretinopathy causing epiretinal membranes and contracture of the retina overlying the graft (patient 11). C, Mild hemorrhage from the graft donor site but a clear graft on the first post-operative day (patient 8). D, This same patient developed a late hemorrhage over the graft (arrow) 6 weeks after surgery. Despite this extensive hemorrhage, patient 8 eventually had an acceptable visual outcome.



Graft Revascularization

ICG patterns of large choroidal vessels were examined 6 months after surgery to confirm the extent to which the free grafts could become revascularised by the residual sub-foveal choroid (Figure 18). In most cases, a new choroidal vascular architecture could clearly be identified as specific to the graft (Figure 18B, D). In contrast, the only presumed complete primary graft failure due to an immediate post-operative hemorrhage (patient 10) had a well-circumscribed area of ICG hypo-perfusion corresponding to the graft (Figure 18F). Choroidal filling of the grafts was also seen on FFA, but revascularization patterns were not obviously correlated to areas of graft autofluorescence (Figure 18).

To examine the vascular properties of the grafts in more detail, high-speed angiography was performed in 4 patients, 3 of whom had a good visual outcome (Figure 19). In patient 4, single frame analysis from high-speed ICG sequence showed a short posterior ciliary artery (SPCA) originating from beneath the graft but filling only the choroidal segment distal to it. Thus sub-foveal choroidal perfusion initially bypassed the graft completely (Figure 19, SPCA1). The graft was subsequently perfused from its periphery by blood entering horizontally through a point of contact with the surrounding macular choroid (Figure 19, SPCA2). We also noted segmental areas of choroidal non-perfusion related to SPCAs that would ordinarily arise from beneath the graft,19 suggesting that these vessels may have been compromised (Figure 19, SPCA3–4).

Visual Outcomes

Changes in visual acuity and logMAR equivalents are documented in Table 43 The mean logMAR vision pre-operatively was 0.82 and fell to 1.16 at the final follow up refraction 6 months after surgery. The 3 patients with no complications (Patients 4, 5, and 8) showed a mean improvement in logMAR visual acuity from 0.88 to 0.79, which has been sustained. Microperimetry was also performed in these patients to assess retinal function over and surrounding the grafts. Patient 8 had good sensitivity over the graft but relatively poor visual acuity at the time of examination (Figure 20A). Conversely, Patient 4 had excellent visual acuity but was unable to see stimuli

projected onto he superior half of the graft (Figure 20B), which corresponded to the non-perfused area seen on ICG (Figure 19C).

The 3 patients who had good functional vision on the graft were also closely monitored beyond the formal 6-month study end point. The vision in patient 5 fell further in the year after surgery and an area of persistent sub-retinal fluid was seen over the graft inferiorly, with a small haemorrhage at the presumed fovea (Figure 21A). No CNV could be identified on FFA, and microperimetry showed poor visual function at this time point (Figure 21B). Fortunately in this patient, the sub-retinal fluid and hemorrhage resolved spontaneously 3 months later, with corresponding improvements in both visual acuity and microperimetry. This was also associated with an improvement in fixation stability and restoration of presumed foveal fixation (Figure 21C, D). The visual acuity in patients 5 and 8 was also maintained or slightly improved beyond the 1-year follow up point.

Figure 18 Autofluorescence images (A, C, D) and corresponding indocyanine green (ICG) angiograms (B, D, E) taken in 3 patients at the 6-month follow-up. Patient 5 has good autofluorescence over the graft (A) and corresponding graft vascular architecture (B). Note that the ICG fills parallel vertical vessels (arrows), which are characteristic of equatorial choroid suggesting successful graft revascularization. Patient 8 has patchy autofluorescence (C), but a clearly graft-specific choroidal vascular pattern (D). Note again the linear arrangement of choroidal vessels within the graft (arrow), which is not normally seen in the fovea. In patient 10, there was autofluorescence from scar tissue surrounding the graft (E), but a corresponding perfusion defect on ICG (F). This patient had a large hemorrhage beneath the graft on the first day after surgery and revascularization is presumed to have failed. See also the corresponding color photographs in Figure 2E.

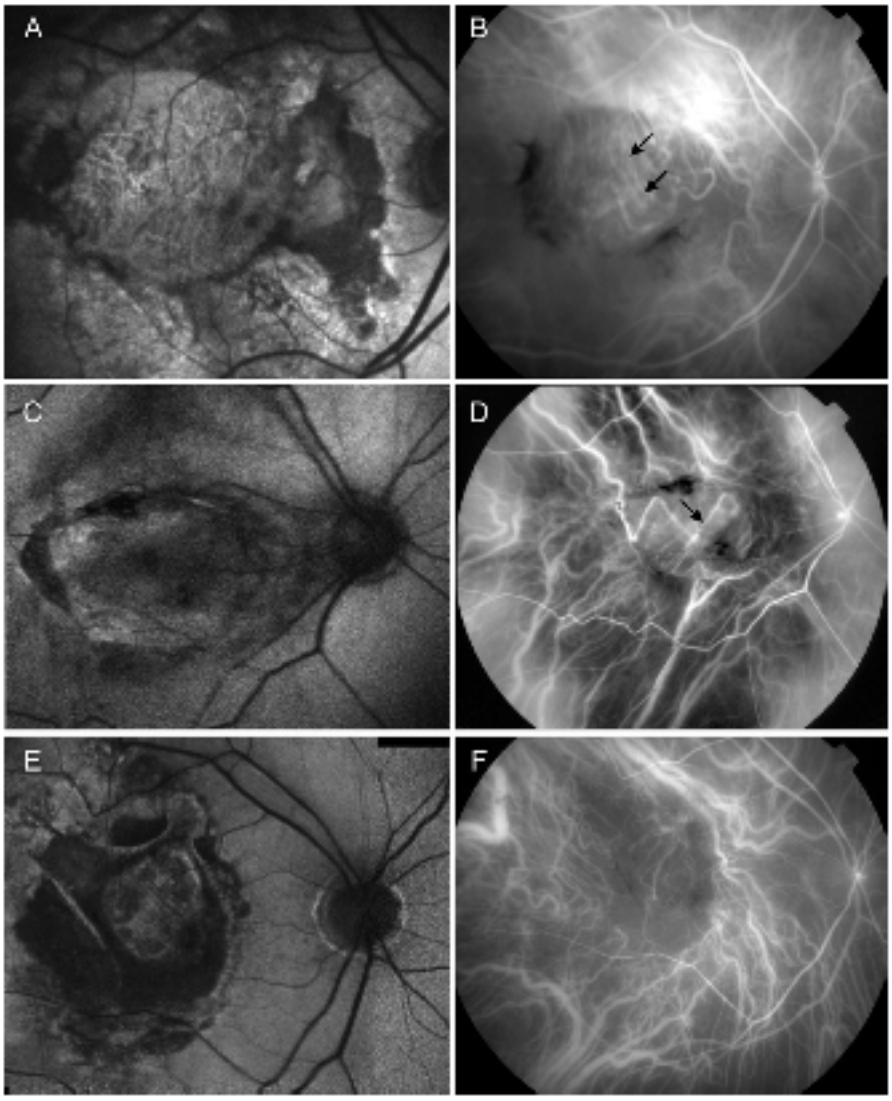


Figure 19 High-speed angiography with indocyanine green (ICG) was used to define the mechanism of graft revascularization in patient 4. A, Pre-operative (pre-op) ICG showing choroidal new vessels (CNV) at the fovea. B, C, Red-free and 6-month post-operative (post-op) ICGs for comparison. Note that the superior half of the graft seems poorly perfused above the arrow in C. D–H, Serial high-speed ICG frames seem to show the graft being perfused horizontally from the residual choroid in contact with the graft inferiorly. The medial posterior ciliary artery (MPCA) fills first (D), followed by the short posterior ciliary arteries (SPCA) 1 and 2 (E). Note that SPCA1 originates from underneath the graft but does not perfuse it. F–H, The graft is perfused from its point of contact with the territory of SPCA2. I, The proposed mechanism of sequential revascularization through the SPCAs is shown diagrammatically. Note also that territories labeled for SPCA3 and SPCA4 fill very late and probably by anastomosis with adjoining territories. These arteries may have been avulsed together with the CNV at surgery or destroyed as part of the original neovascular disease process.

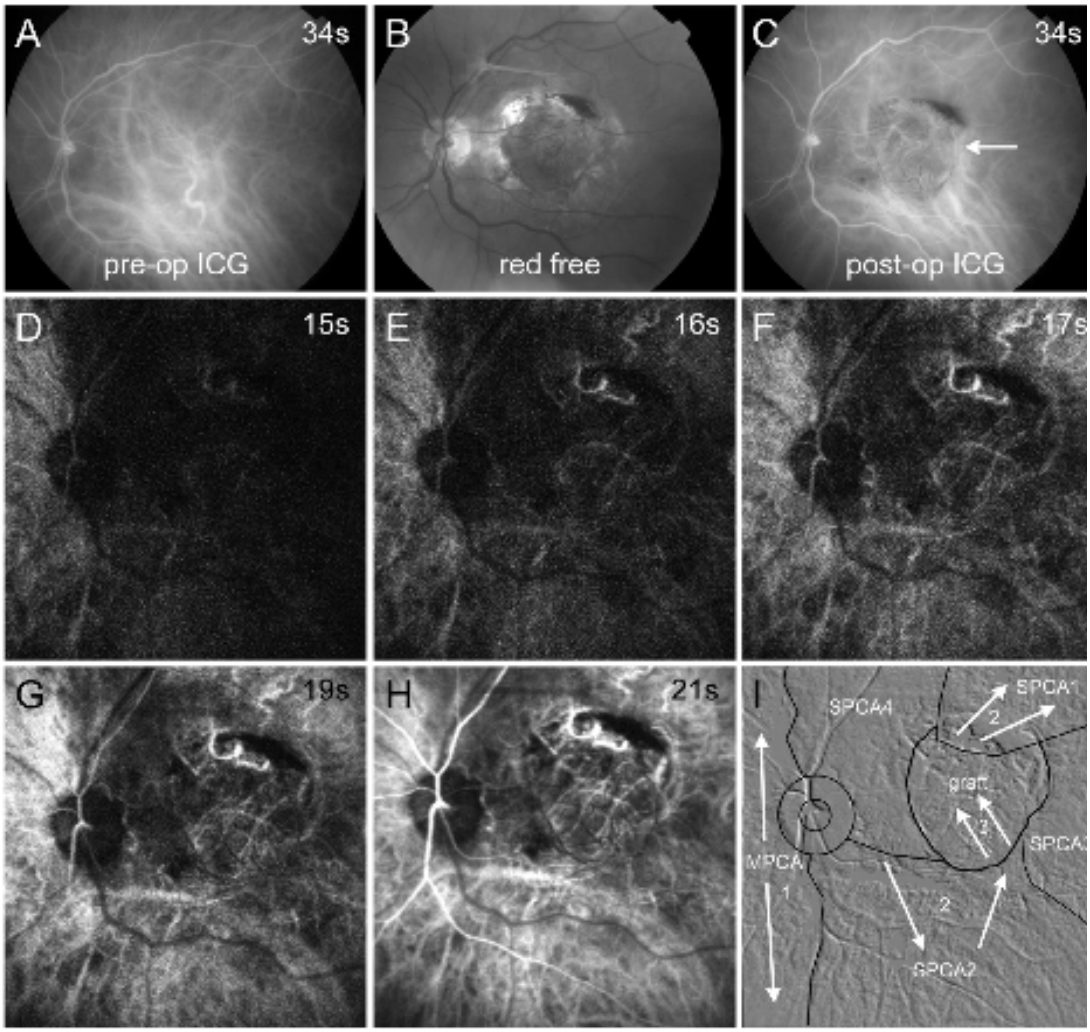


Figure 20 Static microperimetry interpolated maps displaying the range of retinal sensitivity to a Goldmann V stimulus using a Nidek MP1 microperimeter in 3 patients 9 to 12 months after surgery. A, Patient 8 has good retinal function extending over almost all areas of the graft but only modest visual acuity. B, Patient 4 has retinal function limited to the inferior half of the graft but excellent visual acuity. Note the close correlation between retinal sensitivity and blood flow through the graft in this patient in Figure 6. C, A large absolute scotoma over a nonfunctional graft is shown as a negative control from patient 11. D, The relative attenuation range for this machine in decibels (dB) is shown against the false color scheme in the key. Green squares, normal retinal sensitivity through to bright red, which represents appreciation only of the brightest stimulus; dark red areas and hollow squares, absolute scotoma.

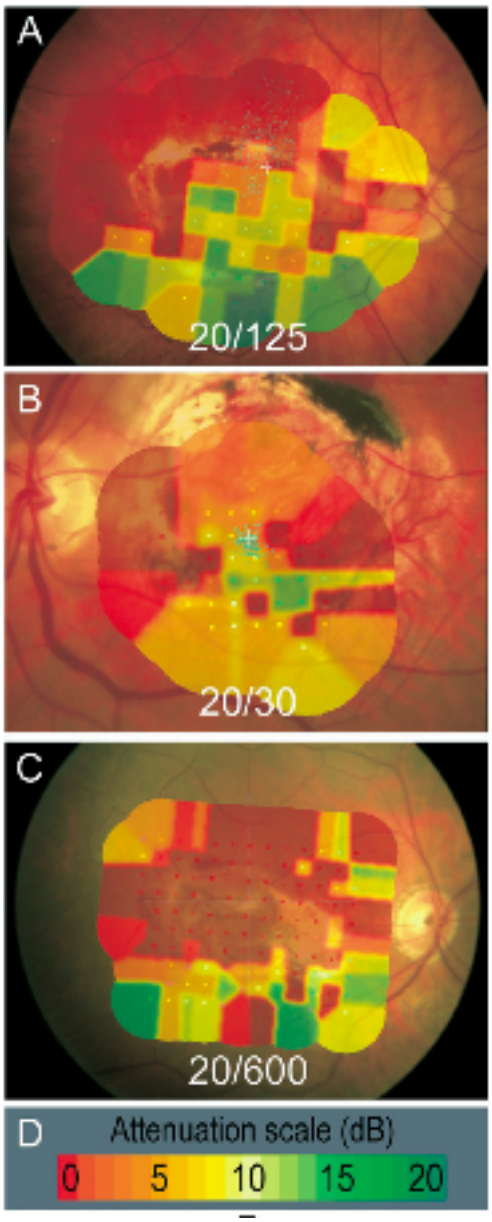
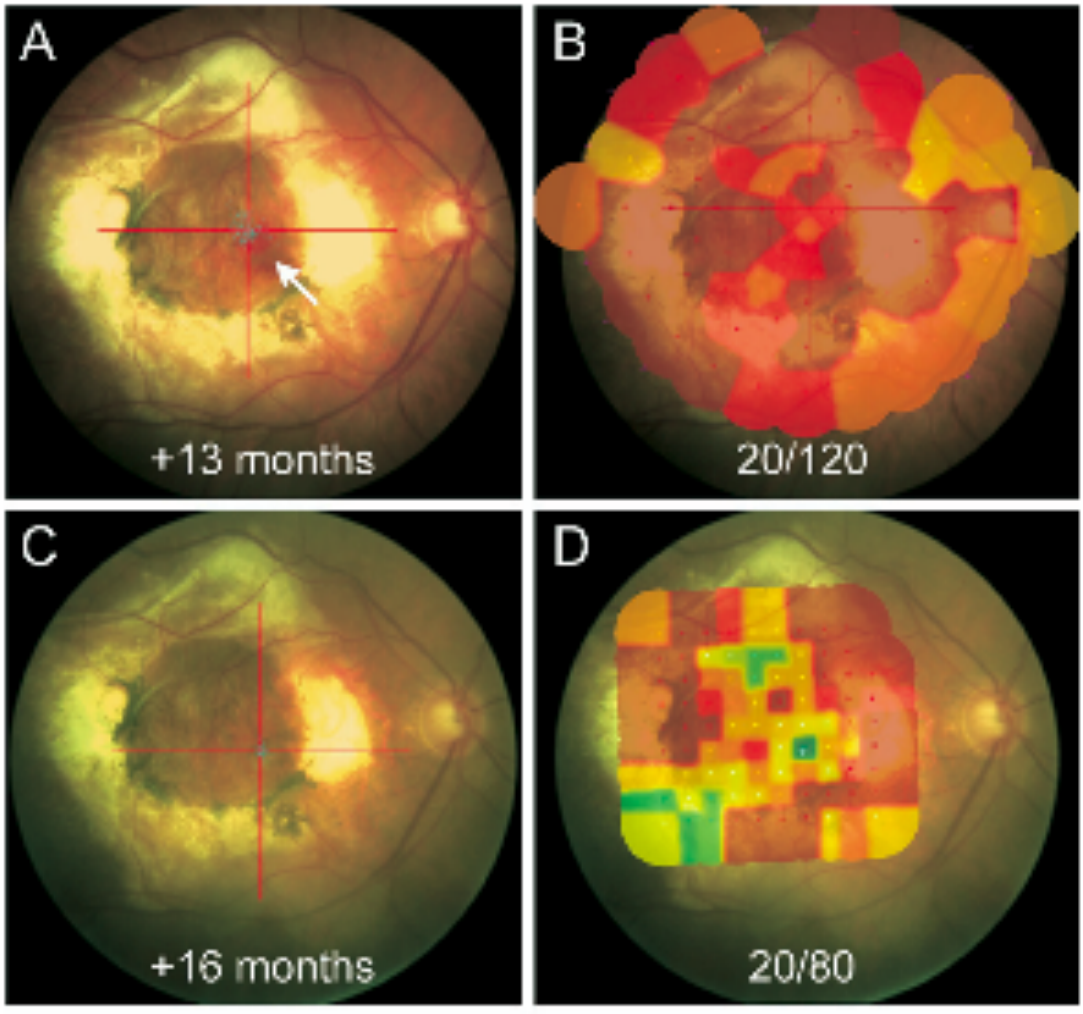


Figure 21 Microperimetry showing progressive improvement in fixation pattern and retinal sensitivity in patient5 between13 and16 months after surgery. A, Presumed extra-foveal fixation due to a small hemorrhage (arrow) associated with sub-retinal fluid. Fixation was relatively unstable at this time (61% of points within 2 degrees of the baricenter of fixation). B, Corresponding interpolated map. C, By 16 months, the hemorrhage and sub-retinal fluid had reabsorbed spontaneously and was associated with much improved fixation stability (98% of fixation points within 2 degrees) and presumed restoration of foveal fixation. This corresponded to improved retinal sensitivity over the graft and visual acuity (D). No choroidal new vessels were detected in this and 2 other grafts showing transient focal areas of leakage. See Figure 7 for key to color codes.



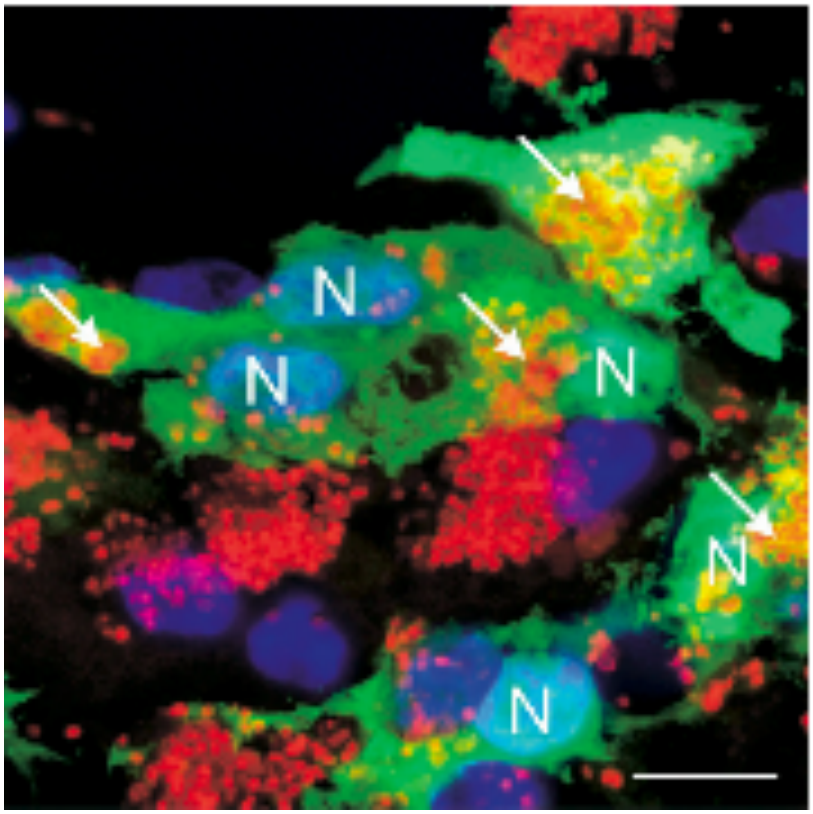
Ex Vivo Gene Transfer

On of the study aims was to see if an RPE choroid graft could be genetically modified outside the eye during the transplantation procedure because this could provide adjunctive molecular therapies targeted to the sub-foveal RPE. This was achieved using a self-inactivating lentiviral vector expressing eGFP under control of a viral promoter (Figure 22), which is known to transduce the RPE highly efficiently. Using a logarithmic dilutional series, identified that $1.5x10^9$ viral transducing units per milliliter in 20 μl of DMEM was the minimum concentration of virus that could transduce rat RPE after incubation at 37° C for 20 minutes. The same protocol was subsequently applied to small portions of human RPE removed from the eyes of 3 patients during RPE choroid grafting. This yielded successful transduction of approximately 10% of cells along the tissue surface, as evidenced by the appearance of GFP after 3 days in culture. Transduction of RPE was further confirmed histologically after 1 week in culture, which showed co-localization of lipofuscin granules in transduced cells (Figure 23). After 20 minutes of immersion in the vector suspension and washing 3 times in culture medium, 3 RPE choroid specimens were co-cultured in contact with non-transduced tissue. In all cases, GFP expression was seen only in the specimens directly exposed to the virus, confirming that the washing procedure was sufficient to render the transduced tissue non-infective under these conditions.

Figure 22 Construct diagram of the reverse transcribed double-stranded DNA generated by the self-inactivating lentiviral vector used for ex vivo gene transfer in this study.

CMV	Cytomegalovirus Promoter
DLTR	Deleted Long Terminal Repeat
eGFP	Enhanced Green Fluorescent Protein Reporter Gene
PBS	Primary Binding Site
PPS	Polypurine Tract;
Ψ	Packaging Signal.

Figure 23 Ex vivo transduction of the retinal pigment epithelium as evidenced by green fluorescence co-localized in cells laden with red autofluorescent lipofuscin granules (arrows) after 1 week in culture. The nuclei of transduced cells are also identified (N). Scale bar, 25 μm.



Discussion

At the time of writing, this study represents the largest published prospective case series assessing the role of autologous transplantation of the RPE in the management of AMD, and the second only after its original description by van Meurs (van Meurs and Van Den Biesen, 2003, van Meurs, 2005). Our angiographic observations provide evidence that a small patch of RPE choroid can become revascularised when transplanted as a free graft into the sub-retinal space after CNV excision. Furthermore, visual acuity was maintained or improved by grafting in 3 of 12 patients (25%) and sustained for at least 1 year. Microperimetry confirmed retinal function over sections of the grafts in these patients, all of whom also commented that they had experienced a subjective improvement in their vision.

Graft survival, assessed by bi-microscopy, autofluorescence, and OCT, was seen in 11 of 12 patients. Electron microscopy of 1 specimen, however, suggested that the quality of RPE cells on the grafts might be variable, although Bruch's membrane seemed consistently intact. It was not possible to determine whether this occurred during separation of the overlying neurosensory retina or during subsequent intraocular instrumentation, and this might require further investigation.

One of the questions posed by the study was whether graft reperfusion occurred vertically, from the choroidal base of CNV excision, or horizontally, through peripheral contact with relatively healthy choroid. The macular choroid is perfused by about 6 to 8 SPCAs that radiate out from the posterior pole and normally fill just after the medial posterior ciliary artery (Hayreh, 1975). Although not conclusive in all patients, it did seem on one high-speed ICG that the graft was reperfused horizontally through contact with surrounding choroid. Graft reperfusion from the periphery may occur because AMD leads to a fibrotic avascular scar beneath the fovea, but the extra-foveal choroid remains highly vascular. Conversely, a recent preliminary study in pigs suggested that graft revascularization can occur from underlying healthy choroid (ARVO abstract 2005:4143). Confirming the horizontal mechanism of graft reperfusion is critical, because it would determine whether or not grafts should be placed in peripheral contact with residual choroid. Conversely, the

technique of laser ablation of the graft undersurface as proposed by Bindewald et al (2004) (Bindewald et al., 2004) would probably only facilitate reperfusion if it occurs from the base.

The high rate of PVR compared with other vitreoretinal procedures may be due in part to the cutting and manipulation of the RPE choroid and consequent dispersal of RPE cells throughout the vitreal cavity. Bare choroid is also left exposed at the donor site, which may release further RPE cells during the post-operative period, particularly as the large defect was sited adjacent to the vitreous base. Posterior PVR in this study was common, and this may further contribute to the high incidence of retinal detachment because the retinotomy site was not routinely lasered, in keeping with usual practice in sub-macular surgery (Hawkins et al., 2004b). In this case, however, the insertion of the graft would further widen the retinotomy and might physically elevate it from the underlying RPE in the initial post-operative stages, during which there would almost certainly be impaired RPE absorption of sub-retinal fluid. This would make the posterior pole highly susceptible to detachment, even without PVR traction.

Early post-operative haemorrhage developed in 4 patients within the first week. This could be problematic if it spread between the graft and underlying macular choroid, because it might prevent revascularization and lead to avascular necrosis. Despite this, complete failure of graft revascularization occurred in only 1 patient. Nevertheless, the visual outcome was poor in all cases of early post-operative haemorrhage; one explanation for this might be that sub-retinal hemorrhage is particularly toxic to the neuroretina. This is partly supported by the results of the Sub-macular Surgery Trials, which showed that hemorrhage associated with CNV was the only indication for which surgery was beneficial in preventing severe visual loss (Bressler et al., 2004). With regard to haemorrhage, the technique described in this study is disadvantageous compared to macular translocation surgery for 2 principal reasons. First, by working through a retinotomy, it was not possible to apply cautery directly to the vascular bed after CNV excision. Also, the presence of a free graft that subsequently became vascularised indicates that angiogenic mechanisms must be active in the sub-retinal space during the post-operative period.

As a vitreoretinal procedure, autologous transplantation of the RPE is unique in that retinal tissue is dissected free from surrounding ocular tissues and might therefore be temporarily removed from the eye during surgery. This provides a potential opportunity to modify the graft genetically within the time frame of the operation, which might be used, for instance, to express angiostatic genes specifically within the sub-foveal RPE (Balaggan et al., 2006). The ability subsequently to render the graft non-infective (by washing off vector suspension ex vivo) may also have advantages over direct sub-retinal administration because vector spread would be more controlled. Gene transfer to the graft might also be combined with gene replacement for inherited retinal diseases that cause macular RPE loss owing to deficiencies in RPE-specific enzymes (Thompson et al., 2005). We have shown that gene delivery to the RPE and transplantation might be combined in a single operative procedure.

The results of this study show that the transplantation of a free graft of equatorial RPE choroid can improve vision in some cases of acute neovascular AMD. The grafts can revascularise and support stable fixation for up to 18 months when transplanted into the sub-retinal space. Further surgical refinements are needed to address the complications, and better pre-operative assessment might provide more information on the visual potential of the neurosensory retina before transplantation. Recent developments in molecular treatments directed against vascular endothelial growth factor may transform the clinical management of exudative AMD. Preliminary data, however, suggest that about 5% of patients lose ≥3 lines of vision after commencing anti-vascular endothelial growth factor treatment (ARVO abstract 2006:2959). These patients and those with a mechanical disruption of the RPE, such as a rip, or geographic atrophy may still be suitable for autologous RPE choroid transplantation.

VI RPE Transplantation: Inherited Dystrophy

Introduction

The inherited macular dystrophies are a diverse group of disorders leading to bilateral symmetrical progressive macular degeneration and irreversible central vision loss (Michaelides et al., 2003). Many phenotypic variations have been described and in a growing number of cases the genetic basis has been identified (Michaelides et al., 2005). Despite great variation in these disorders, retinal pigment epithelium (RPE) degeneration is often seen accompanying the primary retinal changes. This RPE degeneration occurs despite many of the mutated genes associated with these dystrophies, such as *peripherin/rds* (Francis et al., 2005), having been identified as photoreceptor specific. In terms of loss of photoreceptor function it has not been clear whether the primary degeneration occurs in the neuroretina, with secondary RPE dysfunction or whether there is a photoreceptor related 'poisoning' of the RPE primarily with secondary photoreceptor dysfunction and degeneration.

Currently these disorders are untreatable and many affected individuals, including children, sustain irreversible loss of central vision. Although with the advent of gene therapy there has been some indication that treatments maybe available for inherited conditions (Acland et al., 2001) these have not translated into ophthalmic clinical practice.

Eckhardt et al described macular translocation, an indirect form of RPE-choroidal transplantation, in a case of vitelliform disease (Eckardt et al., 2004). They showed a small near vision improvement possibly limited due to the late stage of the disease and surgical complications in that case. RPE transplantation using a full thickness RPE-choroidal graft from a peripheral site in the same eye (van Meurs and Van Den Biesen, 2003) has recently been described. The surgical technique in this is similar to that described by van Meurs. This was a development of previous descriptions of

full thickness choroidal RPE grafts (Peyman et al., 1991, Stanga et al., 2001) and extra-macular free pigment epithelial transfer (Binder et al., 2002, Aisenbrey et al., 2006).

The current study was based on observation that in certain cases of macular dystrophy central vision remained good for several decades but then dropped suddenly over a period of months. It is possible this may indicate a moment where the retinal neurons were present but dysfunctional and the RPE had ceased to support the neuroretina. Thus, RPE transplantation at this point might lead to restoration of the retinal function that was present until very recently.

Methods

Patient

A 51-year-old white male presented with macular degeneration that had been slowly progressive over many years. Visual acuity was 20/30 bilaterally at presentation but had declined to 20/120 over the year preceding surgery. Vision declined in the left eye 6 months prior to decline in the right eye. Functionally the patient described alternating between central and eccentric fixation depending on the task. He became unable to read or to play golf, his major pass-times. The study was conducted with full ethical approval as outlined in the General Methods.

Inclusion Criterion and Exclusion Criterion

The patient was invited to take part in the study with informed consent if all the following criteria were met:

Inclusion Criteria

- Vision loss of at least 3 lines over 6 months before study entry.
- No more than 3 months delay between vision loss and surgery.

Exclusion Criteria

- Unfit for general anaesthetic
- Unwillingness to undertake follow-up
- Other ophthalmic pathology precluding complex vitreoretinal surgery
- Inability to give informed consent

Pre and Post-operative Assessments

Pre and post-operative examinations were performed as outlined in the General Methods. The patient also underwent assessment of retinal sensitivity and fixation pattern after 6 months using a Nidek MP1 microperimeter (Nidek Co.,Ltd., Tokyo, Japan) as described in the General Methods and Appendix 2 Microperimetry was repeated after dark adaptation of the patient (2 hours of complete light excluding patching) to assess the position and stability of fixation. Finally multifocal electroretinogram (ERG) were also recorded binocularly according to established techniques described in Bellman et al (Bellmann et al., 2004b).

Surgical Technique

The surgical technique used in this study was modified from that described earlier[6] for age related macular degeneration. Briefly, a 3-port pars plana vitrectomy was carried out with shaving of the vitreous base. The donor area in the superior retina was demarcated with intra-ocular diathermy. The retina was peeled off this area and removed. A full thickness graft was harvested by cutting the RPE, Bruch's membrane and Choroid to bare sclera. This was lifted on a specially prepared spatula (DORC) (Figure 14).

The macula retina was detached via a single retinotomy temporal to the macula, to make space for the graft. The graft was placed under the retina and was then flattened under heavy liquid. The edge of he donor site was lasered and the heavy liquid was exchanged for silicone oil. Two months later a lens extraction and silicone oil removal was performed. All surgeries were performed by a single surgeon (LDC).

Results

Surgery

Transplantation was performed without complication.

Distance and Near Visual Acuity and reading

Pre-operative distance vision was log MAR 0.8 with a + 0.2/-0.25 x 20 over refraction with a contact lens (-9.50/-0.50 x15) *in situ*. Post operatively with a spectacle correction of –1.00 sphere the logMAR vision was 1.1.

Pre-operative reading speed was 0 words per minute using the standardised MN read test. Post-operatively the reading speed was 56.8 wpm at logMAR 0.6. Reading speed increased as the print size decreased. Higher reading speeds were achieved when the print size approximated and became smaller than the optimal functional area over the graft as shown by the microperimetry (Figure 25).

Colour Fundus Photography and Angiography

The fundus photograph reflects the bi-microscopic examination demonstrating a densely pigmented sub-macular graft *in situ* (Figure 24). Fluorescein angiography in the arterial phase demonstrates early and complete masking of the background choroidal fluorescence by the graft while the surrounding area of atrophic retina manifests a clear window defect. As the run continues there is greater fluorescence of the graft with equal intensity to the background fluorescence of perfused normal retina-choroid (Figure 24)

The ICG post-operative pictures show a different choroidal vessel pattern within the area of the transplant. These vessels are perfused and fill early. Outside of the transplanted area the post-operative vessels pattern is the same as the pre-operative vessel pattern.

Scanning Laser Ophthalmoscope (SLO) Autofluorescence

Pre-operative SLO autofluorescence demonstrates loss of signal in the area of the macular dystrophy. Six months post operatively there is strong SLO autofluorescence from the graft. The fluorescence is of the same intensity, qualitatively, as the areas of normal RPE.

Automated Microperimetry and Fixation Pattern Evaluation

Automated fixation pattern assessment 6 months post-operatively demonstrated that 100% of the fixation points were located at the fovea and within 2º of the gravitational centre of all points over the 30 second test period (Figure 25b). This indicates high quality, central fixation – defined as greater than 50% of the preferred fixation points located within a 2º-diameter circle centred on the fovea) and stable fixation - defined as greater than 75% of fixation points located within a 2º-diameter circle centred on the gravitational centre of all fixation points.

Visual thresholds to a Goldmann V stimulus were within the normal range over some section of the graft temporally and in a small area centrally corresponding to fixation (Figure 25d, inset). However, stimuli were not perceived in large areas of retina over the graft. Visual sensitivities and the area of stimuli perception were greater following dark adaptation of the patient as described previously.

Multifocal ERG

Multifocal ERG was carried out pre- and 8 months post-operatively. The post-operative right eye recordings were significantly improved in the ring of traces from retina over the graft (group A, Figure 26). The average amplitudes of the traces are shown in Figure 26. There was no increase post-operatively in any ring outside of the graft or any ring in the left, un-operated eye.

Figure 24 24a. Pre-operative fundus colour photograph of the right eye showing the macular dystrophy and the area of RPE the unchanged in adjacent un-grafted areas. atrophy. **24b**. Post operative picture showing the pigmented graft in situ. **24c**. Scanning laser ophthalmoscope RPE autofluorescence of the graft showing qualitatively the same fluorescence as normal peripheral RPE and contrasted area of un-grafted RPE loss with no autofluorescence. **24d**. Late stage fluorescein angiogram showing masking of background choroidal flush by the graft but an internal fluorescence suggestive of perfusion within the graft. Pre-operative e and post-operative f indocyanine green angiograms showing the new perfused vessel pattern in the graft (arrows). Note the choroidal vessel pattern is

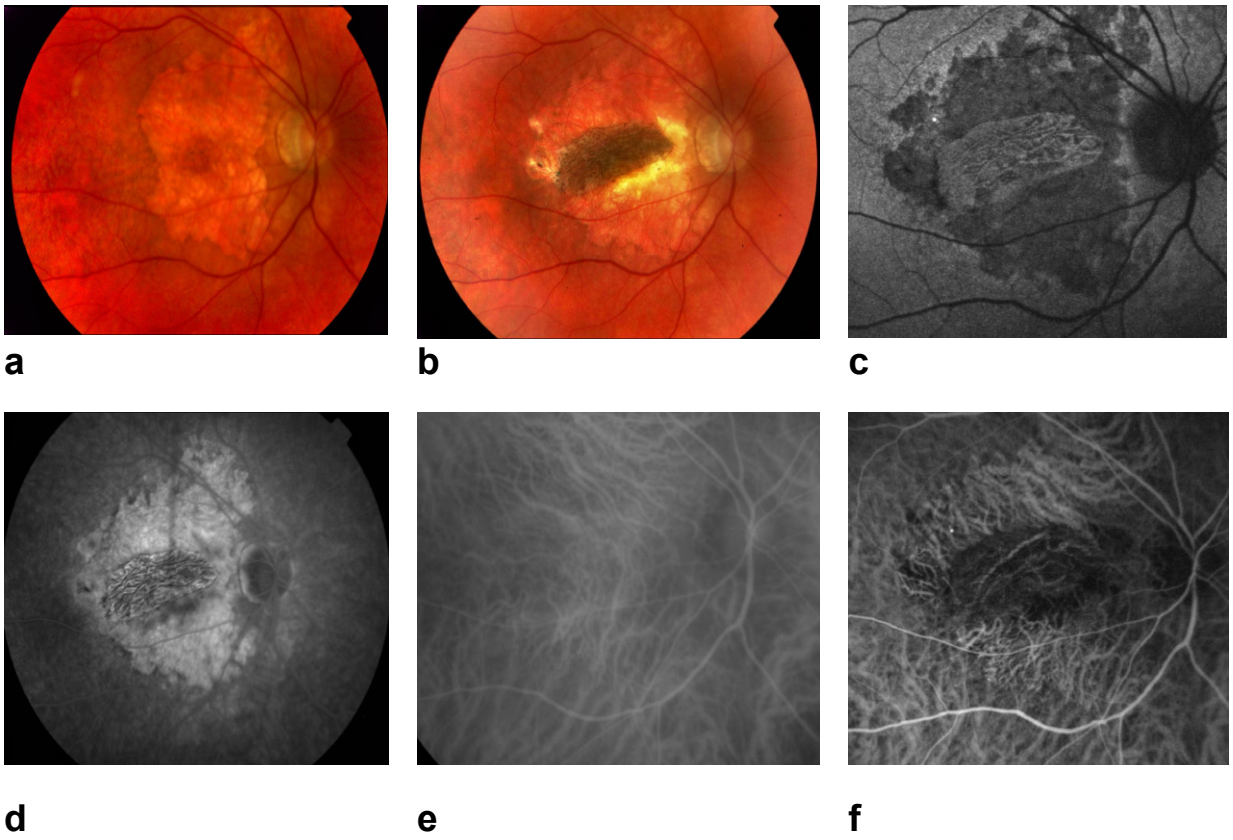
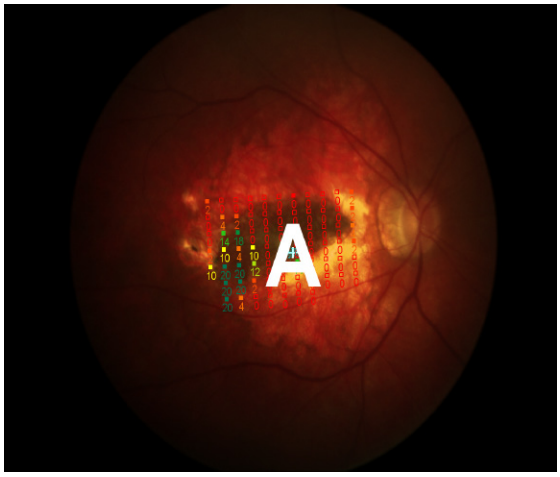
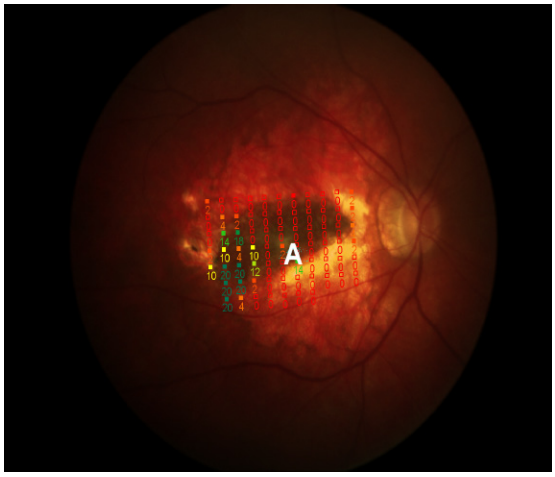


Figure 25 Microperimetry combined with reading speed showing the increasing reading speed as the letter size reduces and approximates the functional area of the graph. a. The size of a 1.3 logMAR letter shows it falling on areas of non-functioning retina. b. The size of a 0.6 logMAR letter showing the peak reading speed at the point where the letter approximates the area of central function. c. The reading speed versus letter size. The points illustrated in a and b are shown with a red arrow. d. A magnified image of the graft and microperimetry (following dark adaptation and using a Goldman V stimulus size and a 1 degree fixation cross) showing the thresholds, colour coded and numerically (the green areas and high numbers approximate normal visual sensitivities). The inset shows the thresholds for the central fixation area in greater detail.



a



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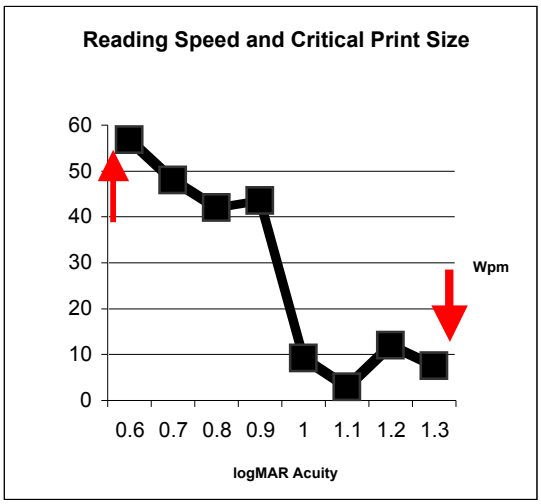
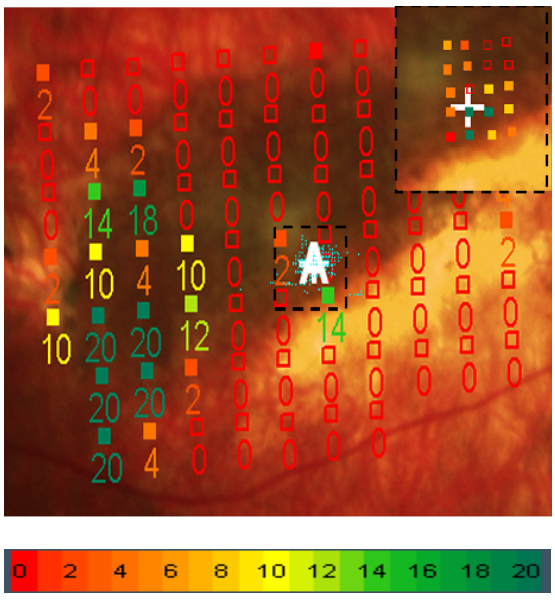
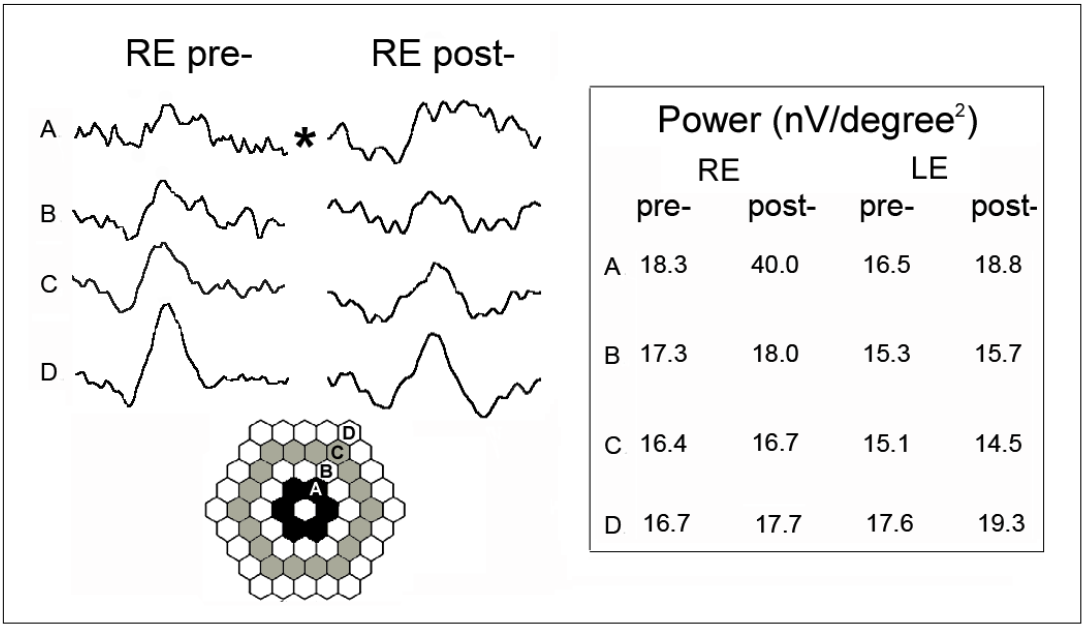


c



d

Figure 26 Multifocal ERG analysis using summated responses from concentric rings of increasing eccentricity to represent different retinal areas. The para-central ring A, shown dark in the insert, corresponds with the area of transplant. The right hand columns contain the mean power values for each ring pre- and post-treatment in nV/deg^2. The power of the responses from ring A from the right eye, indicated by the asterisk, more than doubles following treatment, but there is no significant change in the remaining rings, B-D. None of the left eye rings show significant change post-operatively.



Power (nV/degree^2)

	RE		LE	
	pre-	post-	pre-	post-
A	18.3	40.0	16.5	18.8
B	17.3	18.0	15.3	15.7
C	16.4	16.7	15.1	14.5
D	16.7	17.7	17.6	19.3

Discussion

This study demonstrates a successful transplantation of full thickness RPE/Bruch's and choroid in a case of recent vision loss in the second eye of a patient with progressive inherited macular dystrophy. The results after surgery demonstrate a restoration of retinal function and partial restoration of reading.

Viability of the graft was demonstrated by the presence of the pigmented cells and the autofluorescence 9 months after the graft was completed. The choroid was perfused as demonstrated by a choriocapilliaris flush in the fluorescein angiogram and the presence of a new and distinct pattern of choroidal vessels in the graft footprint on ICG angiography. Presence of a perfused choriocapilliaris is required for normal foveal function as this is its only blood supply. Full field ERG in this patient had suggested that the dystrophy was limited to affecting the central retina and that the peripheral RPE that was transplanted was functioning.

The return of reading function was marked with no reading ability recorded pre-operatively. The reading speed increased with decreasing letter size – a feature contrary to normal reading patterns but consistent with the size and location of the functioning areas of retina as shown by microperimetry (Figure 26.). As peak reading speed was consistent with the size of the functioning retina this is an independent support of the graft being the source of the improved reading vision. The patient also described that he was able to return to playing golf at his local golf club, as it only required a small field to see the ball and the red flag. This is in keeping with the improved reading speed as being associated with effect of the graft on retinal function.

The distance acuity decreased post operatively contrary to the other parameters showing improved function. This may be due to the test protocol commencing with large letters that are difficult to locate on the small area of central retina that had improved function. The test does not proceed to smaller letters if the larger ones are not completed. This will remain a problem for testing of distance acuity in cases

where the graft and rescued retinal functional area are small. The non – grafted areas of central retina would have continued to degenerate and may account for the decline between pre and post-operative distance acuity for essentially larger letters. The improvement in near vision with worsening of distance vision was also described following translocation (Eckardt et al., 2004).

Multifocal ERG demonstrated that there was a significant improvement (more than doubling of the amplitude) in retinal function only in the area of retina over the graft. None of the other concentric rings showed an increase in the same or fellow eye. Once again this powerfully associates the improvement with the graft, as there is both an internal control of the same eye and the lack of change in the equivalent area in the fellow eye.

This study describes the first successful RPE/choroidal graft in a case of inherited macular dystrophy associated with significant improvement of visual function and stabilization of retinal degeneration. It is possible that if the transplants are carried out earlier and the quality of the RPE is improved then this may provide a treatment for a currently untreatable group of blinding conditions.

VII Conclusions

The end stage of a number of macular diseases is irreversible central visual loss, secondary to or associated with loss of support for the neurosensory retina. In the case of exudative AMD profound anatomical disruption of retinal architecture may also be observed. Whilst the current generation of anti-VEGF medications are directed at treating the end stage of the vascular process, the CNV, they are ineffective when large sub-retinal haemorrhage, significant pigment epithelial detachments, and rips of the RPE complicate CNV. Furthermore, in non-exudative AMD currently no medical treatment is available to counter the loss of RPE support, particularly in geographic atrophy. Finally, a number of macular dystrophies are also characterized by loss of RPE support occurring with photoreceptor dysfunction. Under all these circumstances there is disruption of the choriocapilliaris-Bruch's-RPE-photoreceptor axis. This demands a physical restoration of retinal anatomy in order to attempt to recover function and alter the natural history of these disorders. In addition, this restoration needs to be undertaken relatively early in the disease process when photoreceptor loss is limited and visual loss may be secondary to reversible factors such as blood and fluid. This body of work has examined two surgical approaches designed to reconstruct sub-foveal anatomy, macular translocation and RPE transplantation, and gauged the feasibility of these techniques, and their potential in restoring lost function and providing stable long-term vision.

Macular translocation has provided the most compelling evidence for proof of principle that an extra-foveal RPE bed can support photoreceptors. The technique of full 360-degree translocation, as first described by Machemer and Steinhorst (1993), is in effect a functional RPE transplant. In terms of RPE transplantation, MT360 has the advantage that the foveal photoreceptors are placed at a relatively distant position from the original disease bed and onto RPE that is not affected by disease in the case of AMD, it is atraumatic with respect to the choriocapilliaris, Bruch's membrane and the RPE, and there are no issues with regards to revascularization, the size of the RPE transplant, or reestablishing physiologically intact sub-retinal anatomy. MT360 thus represents the ideal full thickness RPE transplant and the outcomes from this surgical intervention represent a benchmark against which other

RPE transplantation techniques can be gauged. A wealth of studies, including the data presented by this thesis, have demonstrated anatomical restoration and recovery of function in AMD, and in CNV associated with myopic degeneration. Furthermore there is evidence that the recovery is maintained in the short (6 months) to medium term (3 years), although a long-term follow up, extending to at least 5 years, will help determine the extent to which this is sustained. Long-term studies also offer an insight into the ability of peripheral RPE cells to continue to meet the metabolic demands placed upon them by foveal photoreceptors, and into the influence of the fovea on the disease process.

In terms of outcomes, the translocation data was comparable with the best-published series and this was also reflected in the complication rates. Of particular note, the data confirmed the premise that one of the principal determinants of successful surgical intervention in macular disease is the number of viable photoreceptors present in the foveal pool at the time of presentation. Early in macular disease, particularly exudative AMD, visual loss may be secondary to factors such as sub-retinal fluid, haemorrhage, exudation, and loss of retinal architecture. Under these circumstances photoreceptors are still viable despite a loss of support and so amenable to being salvaged. With time and disease progression the photoreceptors are irreversibly lost as is this critical window to intervene. The translocation series estimated this window to be approximately 12 weeks from acute loss of vision. This concept underpinned the proposed case selection algorithm, using fixation analysis and duration of symptoms to gauge residual foveal function and photoreceptor viability, as opposed to acuity measures, which can be compromised in the face of viable photoreceptors. This is particularly true of distance acuity, the default outcome measure, which is a poor predictor for outcome when the pre-treatment acuity is less than 20/100.

The data in the thesis supported the case selection algorithm, as some of the best outcomes with translocation were observed in those with the poorest presenting acuity. The algorithm can be applied to any potential surgical or medical intervention for macular disease. In this regard the status of the photoreceptors, as the recipient, is as critical as the status of the RPE, as the donor. When studies of translocation or transplantation are examined closely (extended duration of symptoms with poor presenting acuities) a major factor in poor outcomes is that procedures were performed with a photoreceptor pool that was already at or beyond a critical tipping

point in relation to recovering function. Under these circumstances, any recovery is already compromised, and the surgical intervention itself is likely to additionally degrade the photoreceptor pool, and further limit outcomes. Therefore, to make a complete pre-operative assessment and predict outcomes for surgical intervention necessitates documenting not only distance and near acuities, reading speeds, contrast sensitivity, and an assessment of sub-macular and mid-peripheral RPE (autofluorescence), but also a measure of foveal function by documenting retinal/macular sensitivity (microperimetry/multifocal and full field ERG), symptom duration, and an analysis of foveal fixation, as these are powerful indicators of success.

Additional pre-operative investigations that may assist in predicting outcomes include autofluorescence, angiography and optical coherence tomography. Autofluorescence of the periphery gives an indication of the viability of the donor RPE, and macular imaging provides valuable information regarding pre-existing photoreceptor numbers and the chronicity of visual loss. It has been well documented that in the presence of CNV, autofluorescence decreases with time, reflecting a loss of photoreceptors, RPE, and or disruption of the RPE-photoreceptor axis. Autofluorescence can therefore be a useful tool in helping to assess residual foveal function. Similarly, several investigators have examined outcomes and attempted to correlate these to angiographic subtypes of CNV. In general, lesions that are larger in size, haemorrhagic or vascularised (PED and RAP), and predominantly occult have a worse prognosis. These classifications reflect disease with greater chronicity and extent and so suggest a depleted photoreceptor pool prior to treatment. Finally, recent advances in OCT, particularly spectral domain scanning offer the ability to image macular anatomy in greater detail to gauge the extent of sub-macular pathology, map the RPE pre-operatively, and identify co-pathology such as pre-retinal membranes.

The quality of the restoration achieved with successful translocation was examined with a detailed investigation of the best outcomes from this series. The study demonstrated that peripheral RPE was capable of supporting foveal photoreceptors to the extent of restoring normal retinal sensitivity with a single stable locus of fixation. Moreover the retinal function closely approximated or matched normal age-matched subjects not only in acuity measures but also in measures of reading

performance. The latter was achieved despite translocation being shown to compromise saccadic behaviour when performing reading tasks.

The studies of macular translocation and RPE transplantation conducted in this thesis confirmed the feasibility of these procedures to treat sub-macular disease and surgically reconstruct retinal architecture. While translocation was a far more involved, and longer procedure, the functional outcomes were more favourable than RPE transplantation. This result is a reflection of the complications associated with each procedure, particularly PVR detachments and early haemorrhage, and as mentioned earlier this result can be attributed to the fact that translocation is in fact a very atraumatic form of full thickness RPE transplantation. Despite translocation being a much more effective procedure, it is ultimately limited by its relative complexity, the surgical time required and the consequent impracticalities of meeting an ever increasing service demand, the need for cyclotorsion surgery, and the constraints of treating only second eyes due to the considerable problems of cyclotorsion if undertaken on a first eye. Furthermore, when compared to the modern era of medical therapies, translocation appears an even more cumbersome undertaking. Having said that it is a real and viable option when a salvage procedure is required in the face visual loss secondary to profound mechanical disruption of the retina in a second eye.

In comparison, autologous full thickness RPE transplantation, while being a more straightforward, shorter, and more versatile procedure in terms of manipulation of the graft prior to placement and disease processes amenable to treatment, is susceptible to a high complication rate, particularly PVR. This technique of transplantation results in release of RPE cells into the vitreous cavity at a number of stages from harvesting the patch, to delivery into the sub-retinal location, to continued RPE release from the bare bed of the donor site. Furthermore, the high rate of posterior PVR documented in this study was felt to be due to the widening of the retinotomy required to deliver the graft, combined with the routine practice of not lasering the retinotomy site. All of the aforementioned factors combine to contribute to a high risk of PVR traction and detachment. In addition to PVR, other causes of graft failure include a paucity of RPE cells on the patch secondary to surgical trauma, pre-existing RPE dysfunction, or graft ischaemia. In comparison to translocation, considerable surgical trauma of the graft arises from the physical manipulation of the graft from harvesting to delivery,

and the technique still requires evolution and refinement to minimise disruption of the RPE layer. In the transplantation series, electron microscopy provided evidence of this trauma by demonstrating areas of RPE loss overlying intact Bruch's membrane and avulsion of the apical aspect of RPE cells. The study also showed strong adhesion between the retina and the underlying CNV, particularly with RAP lesions, further contributing to loss of photoreceptors and compromising the success of the graft. In terms of pre-existing disease, pre-operative work up with autofluorescence and full field ERG would ascertain if the peripheral RPE was suitable, especially when the procedure was performed on patients with retinal dystrophies. With regards to graft revascularisation, the transplantation series demonstrated revascularization in the majority of cases. Previous investigators have reported that revascularization occurs via direct contact with the choroid underlying the graft however high-speed ICG angiography conducted in one particular case in this series demonstrated that reperfusion occurs via connection between the edge of the patch and the choroidal bed as per a skin graft. This method of contact revascularization may well be the initial route of perfusion before subsequent underlying vascular remodeling occurs and further investigation is warranted. In terms of failure to vascularise, this is may be due to a failure to establish contact with the choroidal bed secondary to either a physical barrier, such as intervening haemorrhage, or an absence of choroidal vasculature secondary to pre-existing degenerative changes or vascular damage incurred at the time of surgery with CNV removal. As with translocation, long-term studies will help to determine the ability of peripheral patch grafts to maintain foveal function, the incidence of delayed patch ischaemia, the rate of continued RPE and photoreceptor loss over the patch, and the impact of disease recurrence and the effect of anti-VEGF agents on graft perfusion.

For exudative AMD the surgical approaches outlined in this thesis, although capable of restoring the RPE, supporting photoreceptors, and reversing visual loss have shown limited and compromised restoration of overall visual function. This is particularly the case when compared to the current generation of anti-VEGF based therapies. In this regard these surgical approaches, especially RPE transplantation may be better deployed against other disease entities such as non-exudative AMD and macular dystrophies that are associated with the loss of the RPE and choriocapilliaris. In this thesis we presented a case of autologous transplantation for a patient with a progressive inherited macular dystrophy. The surgery restored the RPE support to the photoreceptors with a partial recovery of reading ability and

stabilization of the retinal degeneration. There are a number of potential difficulties with this approach such as the timing of the intervention when the visual deterioration is gradual. Unlike exudative AMD there may be no acute visual loss and so there arises a difficult decision between intervening when vision may well be useful with sufficient photoreceptors to salvage, and when vision declines with too few photoreceptors. Another difficulty is that if RPE degeneration is secondary to primary photoreceptor dysfunction, then RPE transplantation is ineffective. Furthermore, with a retinal dystrophy, all RPE cells may be affected and so autologous grafting will again be of little benefit. In this regard, the future of RPE transplantation may be a return to homologous techniques whereby RPE defects can be overcome by the use of an unlimited supply of artificial RPE lines, deliverable in a reproducible manner, on a stable platform, and without limitation on the size of the defect to be treated. This would also have the added advantage of reducing the surgical time and possibly the PVR rate if the graft does not need to be harvested. As such alternative RPE lines are an area of intense investigation with a number of candidate cell sources. These include immortalized RPE cell lines, retinal progenitor, embryonic stem cells, umbilical cord cells, and bone-marrow derived stem cells (Coffey et al., 2002, Klassen, 2006, Lund et al., 2006, Harris et al., 2006).

Another advantage of homologous transplantation is the ability to modify the RPE either pharmacologically or genetically prior to delivery. The latter was successfully demonstrated in the transplantation series with ex-vivo modification of a graft through transduction of the RPE with a viral vector. This form of graft manipulation provides a method of gene replacement where the delivery is more directed, where the degree of vector spread is limited compared to the current method of sub-retinal injection of vectors, and where there is an opportunity for gene therapy to be combined with other modalities of retinal treatment. Potential directions for this type of therapy include delivering genes for specific inherited retinal diseases as well as treating acquired disorders with genes encoding neurotrophic and angiostatic factors. As previously discussed in this thesis, one of the major obstacles to homologous transplantation however is the rejection process, and overcoming this is a challenge for future research.

The treatment of macular disease has rapidly progressed in recent years with surgical interventions providing a number of therapeutic modalities. Surgery has

advanced from the removal of subretinal pathology, to the reconstruction of anatomy , to being a delivery vehicle for novel adjuvant therapies. Animal and human studies of translocation and transplantation have demonstrated recovery of function in complex disorders such as AMD as well as retinal dystrophies. Both treatments represent RPE replacement, each with their own set of limitations. Of the two approaches RPE transplantation provides the most flexibility with homologous cell based methods in combination with pharamcological and genetic treatments offering the prospect of long-term stable central vision in a range of macular disorders.

Publications

1 Assessment of Reading Behaviour with an Infrared Eyetracker following 360-degree Macular Translocation for Age Related Macular Degeneration
Uppal G S, Feely M, Membrey L, da Cruz L, Rubin G
Submitted to IOVS (Under Review)

2 Reversal of Vision Loss in a Progressive Inherited Maculopathy following Autologous Full Thickness Choroidal - RPE Transplantation
da Cruz L, Uppal G S, Balaggan K S, Neveu M, Milliken A B, Rubin G, Holder G E, Webster A
Submitted to Retina (Under Review)

3 Long Term Outcomes following Full Macular Translocation Surgery in Neovascular Age Related Macular Degeneration
Chen F K, Patel P, Uppal G S, Tufail A, Coffey P, da Cruz
Submitted to CEO (Under Review)

4 Clinicopathological case series of four patients with inherited macular disease.
Wickham L, Chen F K, Lewis G P, Uppal G S, Neveu M M, Wright, Robson A G, Webster A R, Grierson I, Hiscott P, Coffey P, Holder G E, Fisher S K, da Cruz L
Investigative Ophthalmology and Visual Science March 2009 50(8): 3553-3561

5 Long-term Visual and Microperimetry Outcomes Following Autologous Retinal Pigment Epithelium Choroid Graft for Neovascular Age Related Macular Degeneration
Chen F K, Uppal G S, MacLaren R E, Tufail A, Aylward G W, da Cruz L
Clinical and Experimental Ophthalmology February 2009 37(3): 275-285

6 A Comparison of Outcomes Between Macular Translocation and Autologous Retinal Pigment Epithelium-Choroid Patch Graft in Neovascular Age Related Macular Degeneration
Chen F K, Patel P, Uppal G S, Rubin G, Coffey P J, A, Aylward G W, da Cruz L
Investigative Ophthalmology and Visual Science January 2009 50: 1848-1855

7 Evidence of Photoreceptor Function using Microperimetry following Autologous Retinal Pigment Epithelium-Choroid Graft in Macular Dystrophy: Outcome in Five Cases
Chen F K, Uppal G S, Rubin G, Webster A, Coffey P, da Cruz L
Investigative Ophthalmology and Visual Science March 2008 49: 3143-3150

8 A New Algorithm for Assessing Patient Suitability for Macular Translocation Surgery
Uppal G S, Lee J, Milliken A, Acheson J, Hykin P, Tufail A D, da Cruz L
Clinical and Experimental Ophthalmology July 2007 35(5): 448-457

9 Autologous Transplantation of the Retinal Pigment Epithelium in the Treatment of Neovascular Age-Related Macular Degeneration
(Uppal G S & MacLaren R E)[1,] Balaggan K S, Tufail A D, Munro P M, Milliken A B, Ali R R, Rubin G S, Aylward G W, da Cruz L
Ophthalmology March 2007 114(3): 561-70

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Appendicies

Appendix 1 _ Visual Acuity and Contrast Sensitivity

I Retro-illuminated Lighthouse / ETDRS Chart at 4 metres (+ Distance Correction If Worn)

At a visual acuity of 6/60 test the patient from 4 metres. If the visual acuity is worse than 6/60 test the patient from 2 metres.

Patient begins at top of the chart and reads down.

If patient can read 3 letters or more from a line they can try the next line (use criterion free method). If patient fails to read all letters correctly on top line, then restart testing at half distance (minimum 1 metre).

Calculate the total number of letters read correctly (if patient reads O instead of C or vice versa count as correct).

II MN Read™ Charts at 25cm (+ Near Correction If Worn)

The patient reads sentences from the Minesota Low-vision Reading (MNREAD™) cards at a distance of 25cm in daytime northern hemisphere lighting conditions as quickly and accurately as possible. A blank piece of card is used to cover the sentences below the one being read. A note is made of words missed/read incorrectly and time taken to read each sentence.

Reading Acuity

The reading acuity is the smallest print size at which patient can read the entire sentence without making significant errors.

After the patient has read as much of the chart as possible use following formula:

Reading Acuity = 1.6 – (number sentences x 0.1) + (number of word errors x 0.01)

e.g. = 1.6 – (14 x 0.1) + (4 x 0.01)

= 0.24 logMAR.

Reading Speed

The reading speed is the time in seconds taken to read each sentence and recorded as words read per minute (wpm). The reading speed for each print size is calculated using the following formula:

Reading Speed = 600 / time to read sentence (seconds)

e.g. = 600 / 5.0

= 120 wpm

Critical Print Size

The critical print size is the print size at which patients can read with their maximum reading speed. The maximum reading is identified from a plot of reading speeds at each print size. A patients reading speed should remain approximately constant; the print size at which the reading speed deteriorates is taken as the critical print size.

III Pelli-Robson Contrast Sensitivity at 1 metre (+ Distance Correction If Worn)

Contrast sensitivity is measured using the Pelli-Robson contrast sensitivity chart at 1m in daytime northern hemisphere lighting conditions. The chart consists of triplet opto types 20/60 in size, whose size remains constant throughout but whose contrast decreases both down and across the chart. Pelli-Robson scores reflect the logarithm of the contrast sensitivity of each triplet group.

The patient begins at top of the Pelli-Robson chart.

Patients are asked to guess and to keep looking at letters as they sometimes start to appear.

If a patient reads ‘O’ instead of ‘C’ or vice versa count as correct.

If 2 letters of a triplet are read go to next triplet.

Appendix 2 _ Microperimetry

Scanning Laser Ophthalmoscopy Microperimetry

SLO:	Heidelberg Retina Angiograph 2 [HRA2]™, Dossenheim, Germany
Circular Grid:	4 x 4 - 24 Pixel Size
Refraction:	Change to Distance Sphere

Instructions

The operator centres the optic nerve on screen and places a landmark over a recognisable area e.g. next to the optic nerve.

The grid is placed over the macula to include the fixation area.

The patient is asked to fixate on a stationary cross throughout the programme and will see flashes as the programme proceeds. The patient is reminded to continue to fixate on the cross and press a button each time they see a flash:

'When you see a flash I would like you to press this button - Remember all the time to fixate on the cross'

After the programme has run any scotoma detected are mapped out by placing extra targets around the area of reduced sensitivity to delineate the edge of the scotoma.

SLO Microperimetry Measurements

Fixation Stability

Distance and Angle of Fixation from Fovea

Presence and Area of Scotoma

Appendix 3 – Slit Lamp Assessment of Quality of Fixation

Step 1

A 0.2 millimetre slit lamp spot is projected onto the posterior pole of the patient and the patient asked to fix on the target. The response is positive if the patient fixes with the fovea and can maintain fixation for 1 – 3 seconds.

Step 2

The slit lamp stimulus is changed to a 2 mm square and the patient is asked to look at each corner of the square systematically. The response is positive if the patient fixes with a single saccade to each corner of the square and can maintain fixation at each position for 1 – 3 seconds.

Step 3

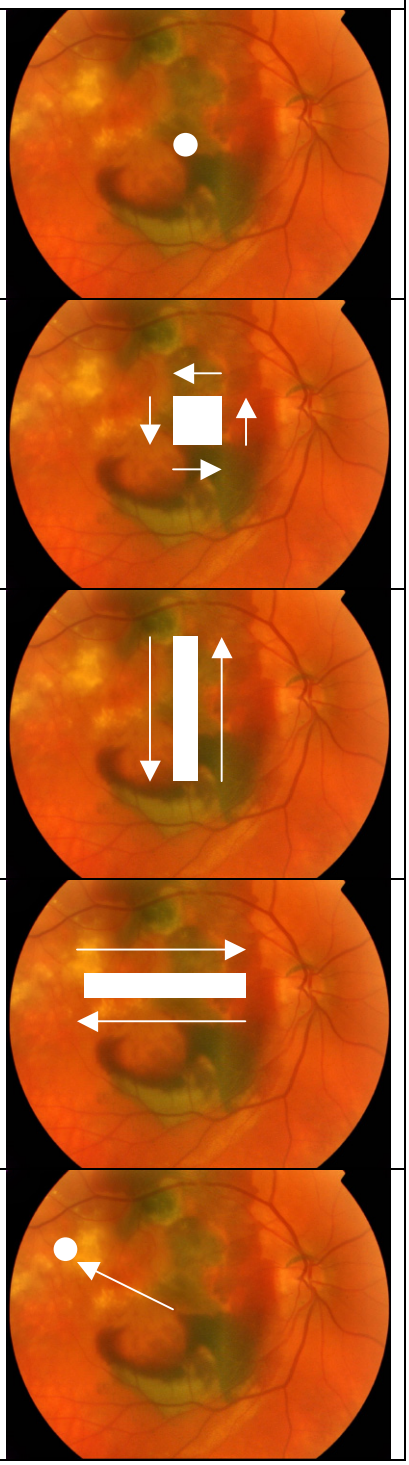
The slit lamp beam is lengthened and narrowed to form a 4 mm X 1mm vertically orientated line. The patient is look asked to look at the top of the line and then the bottom of the line. The response is positive if the patient fixes to the top and bottom of the target with a single saccade and can maintain fixation at each point for 1 – 3 seconds.

Step 4

Step 3 is repeated with the line orientated horizontally.

Step 5

The final task is to place a 0.2 millimetre spot of light at an extrafoveal location and the patient is asked to re-fixate the target. The response is positive if the patient can re-fixate the target with a single saccade and can maintain fixation for 1 – 3 seconds.

Instructions to Patient	Target	Task	Slit Lamp View
Look at the spot of light	0.2mm Spot	Fixes with Fovea + Maintains Fixation 1 – 3 s	
Look at the top right corner of the square Repeat instruction for: Bottom Right Corner Bottom Left Corner Top Left Corner	2x2mm Square	Projects to Each Corner of Square with a Single Saccade + Maintains Fixation 1 – 3 s	
Look at the top of the bar Now look at the bottom of the bar Repeat once	4x1mm Vertical Slit	Fixes to Top and Bottom of Slit with a Single Saccade + Maintains Fixation 1 – 3 s	
Look to the left side of the bar Now look at the right side of the bar Repeat once	4x1mm Horizontal Slit	Fixes to Left / Right of Slit with a Single Saccade + Maintains Fixation 1 – 3 s	
Look at the spot of light	0.2mm Spot Placed Outside Lesion	Re-fixate to Fovea with a Single Saccade + Maintains Fixation 1 – 3 s	

Failed Task:
Defined as failure to follow instructions or maintain fixation for 1 – 3 s as outlined.

Appendix 4 _ EyeTraker™

Data from fixation stability and PRL is used to calibrate eye position for measurements with the EyeLink™ eye tracker (EyeLink™ Gaze Tracking, SMI SensoMotoric Instruments, Berlin, Germany). This system is used to record eye position and direction during reading while allowing binocular viewing and natural movements of the head. Saccadic eye movements and fixation duration during reading will also be measured and quantified with the EyeLink™.

EyeTracker™ (Fixation Stability / Fixation Accuracy Task)

Habitual distance correction

- Leave unaided unless refractive error > +/- 2.00DS or 0.75DC
- If habitual correction is bifocal or varifocal then distance correction in trial frame

BUT

- Leave unaided unless refractive error > +/- 2.00DS or 0.75DC

EyeTracker™ (Reading Task)

+2.00DS addition over distance correction in trial frame ignoring small cyls (<0.75DS)

Seat patient **50cm** from EyeTracker™. Set resolution to 800x600 pixels.

Calibrate with 3 dots. Validate (accept green or amber).

- Fixation stability programme x 2 (A&D)

Instructions:
You will see a dot on the screen. I want you to fixate this dot, that is, focus on the centre of the dot as best you can and follow it when it moves.

- H Sacc (B)

Again you will see a dot on the screen. This time the dot will move on the screen from the centre to the left or right and then back again. I want you to fixate the dot as closely as you can.

- Vertical Sacc (C)

This time the dot will move from the centre and up or down and back again.

- Sentences (E) – use critical acuity level from MN Read cards or ReadNavigator

Read out loud as quickly and accurately as possibly, try not to correct yourself. Make a note of the number of errors made.

- EU texts (F) – use critical acuity level taken from MN Read cards or ReadNavigator

Read out loud as quickly and accurately as you can, if you make an error try not to go back and correct yourself. Make a note of the number of errors made.

EyeTracker™ Measurements

Fixation Stability
1. Number and sum of PRLs

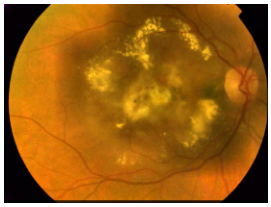
Eye Movement – Horizontal and vertical saccades
1. Number of saccades taken to find target
2. Latency up to first eye movement
3. Peak velocity value of fastest saccade

Sentences
1. Number of forward saccades
2. Percentage of total saccades which are regressive
3. Average of number of saccades to find beginning of each line of Sentence
4. Number of words read per minute

Appendix 5_Macular Translocation: Pre-Operative Images

Pre-Operative Data: Macular Translocation Patient_1

Colours / Red Free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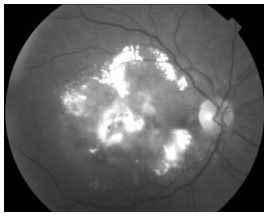

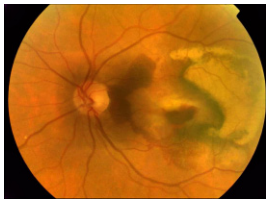
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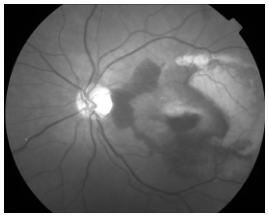
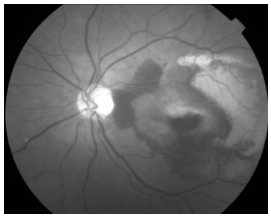
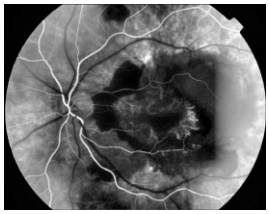
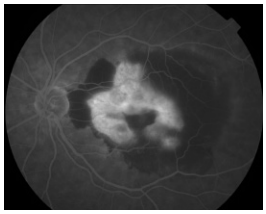


FFA

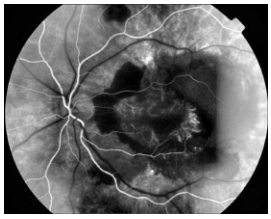

Red Free

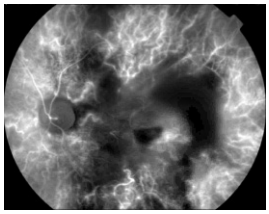

Early


Mid


Late

ICG


Early FFA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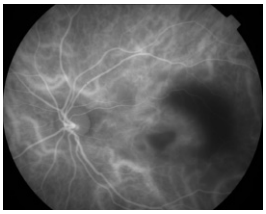
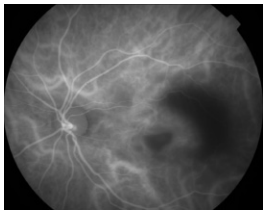
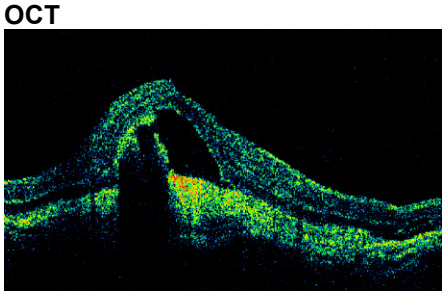

Mid


Late

OCT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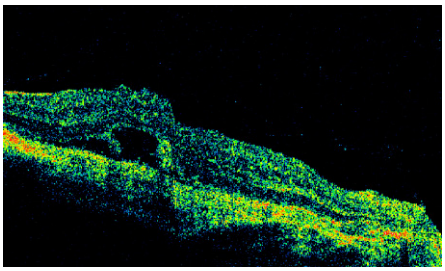
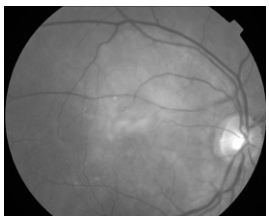

OS

Pre-Operative Data: Macular Translocation Patient_2

Colours / Red Free


OD

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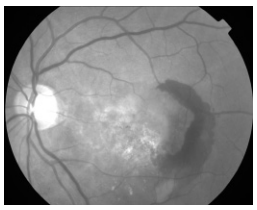
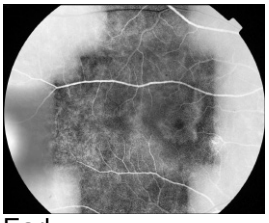
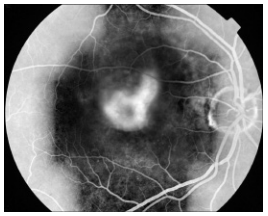


FFA


Red Free


Early


Mid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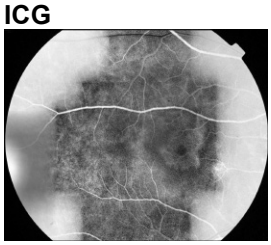
ICG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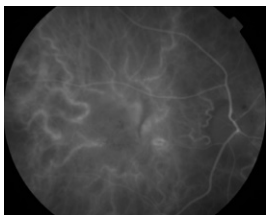
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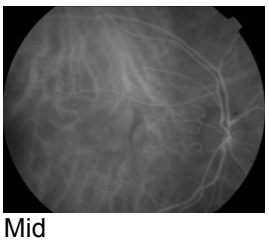
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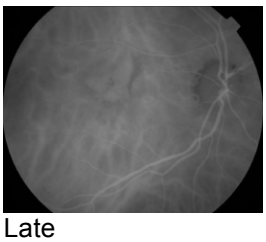
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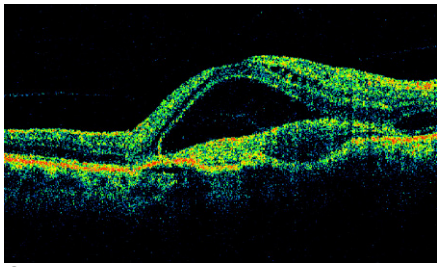
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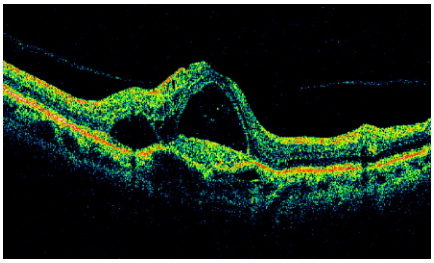
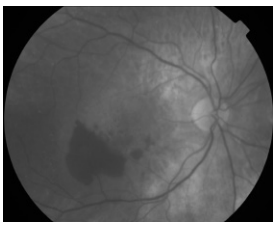
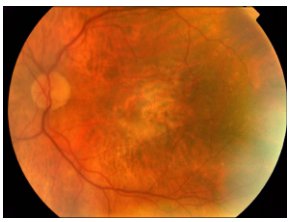

OS

Pre-Operative Data: Macular Translocation Patient_3

Colours / Red Free


OD

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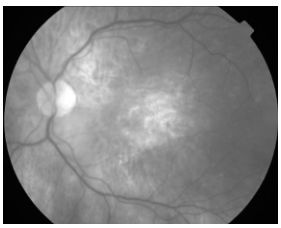
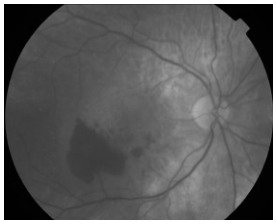
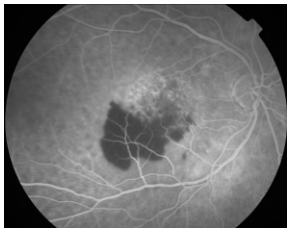


FFA


Red Free


Early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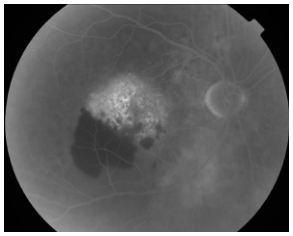
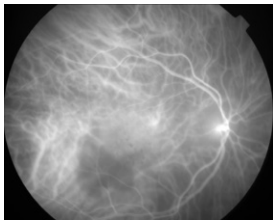

Late

ICG


Early FFA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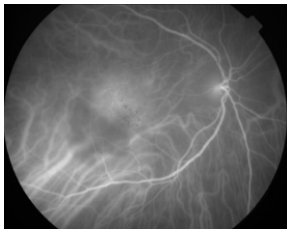
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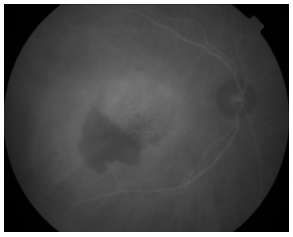
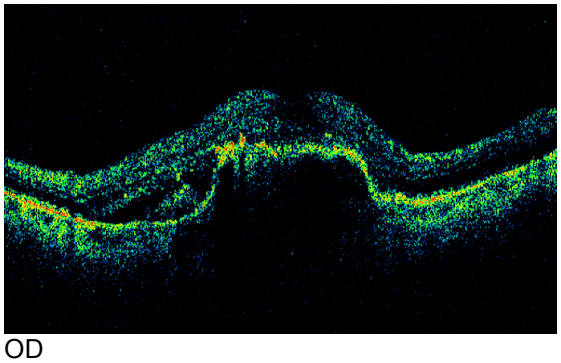

Late

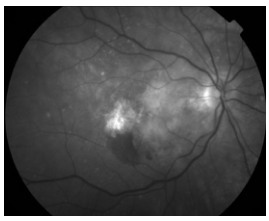
OCT


OD

Pre-Operative Data: Macular Translocation Patient_4

Colours / Red Free


OD

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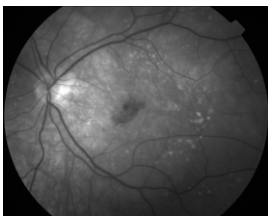
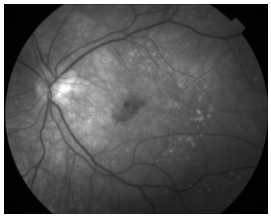
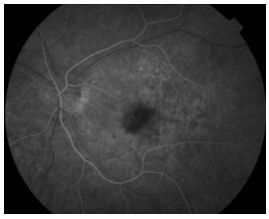
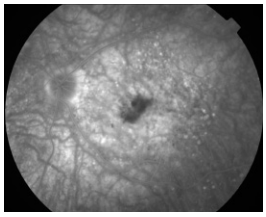


FFA


Red Free

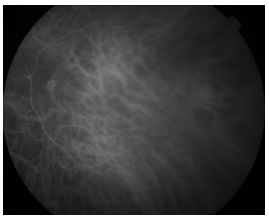

Early

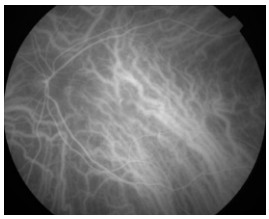

Mid


Late

ICG


Early FFA


Early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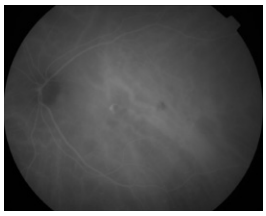
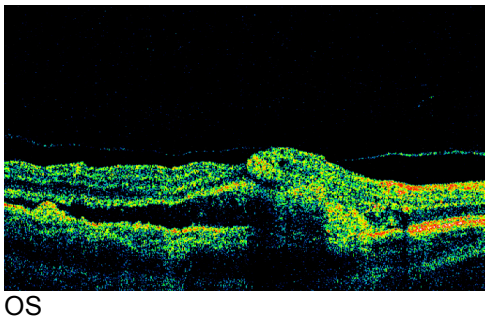

Late

OCT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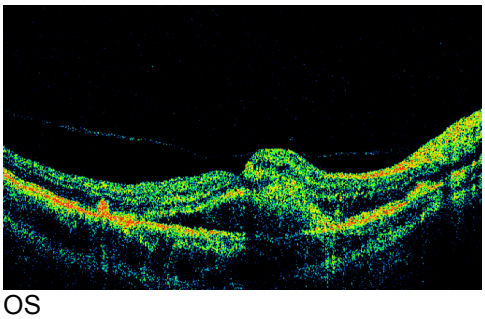
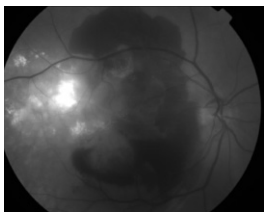
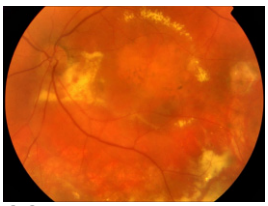

OS

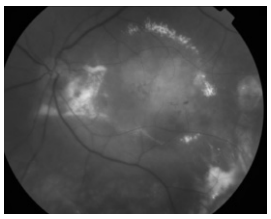
Pre-Operative Data: Macular Translocation Patient_5

Colours / Red Free

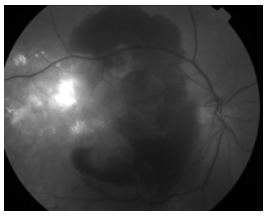

OD

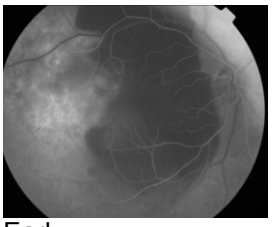


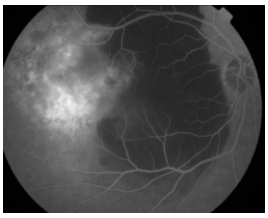

OS

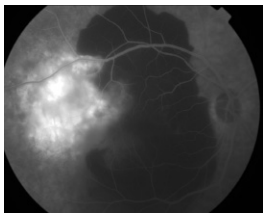


FFA

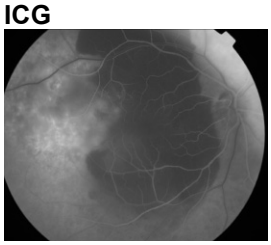

Red Free

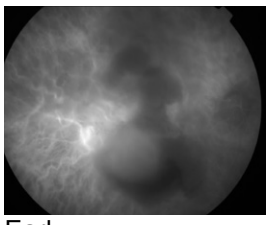

Early

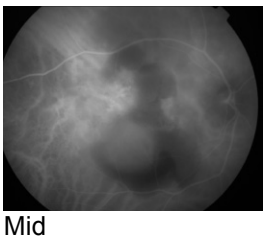

Mid

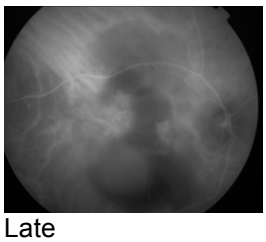

Late

ICG

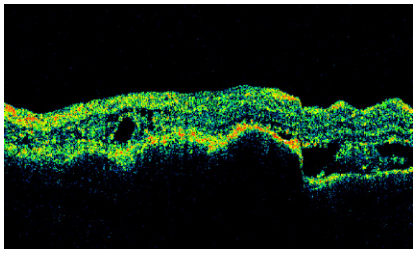

Early FFA


Early

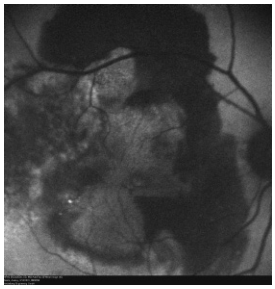

Mid


Late

OCT


OD

SLO

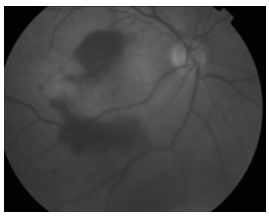

OD

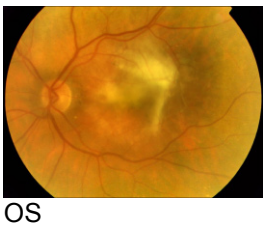

OS

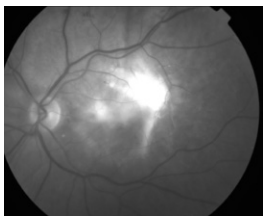
Pre-Operative Data: Macular Translocation Patient_6

Colours / Red Free


OD

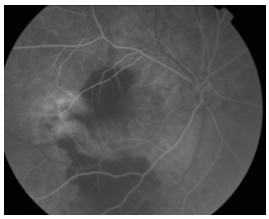


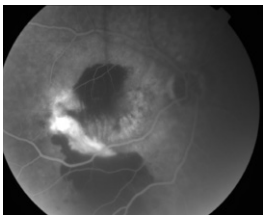

OS

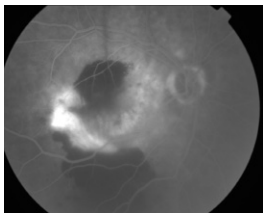


FFA

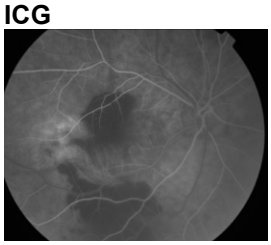

Red Free

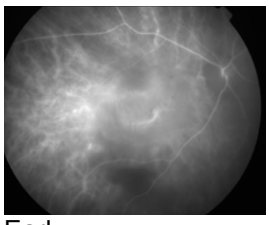

Early

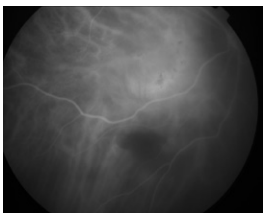

Mid

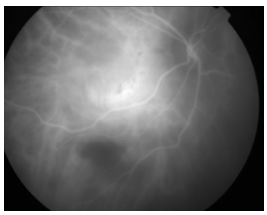

Late

ICG

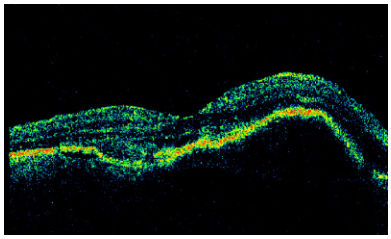

Early FFA

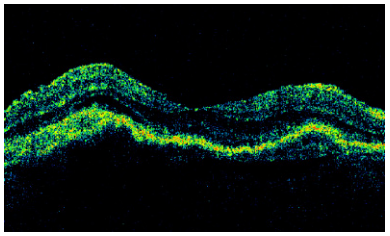

Early


Mid


Late

OCT


OD


OD

SLO

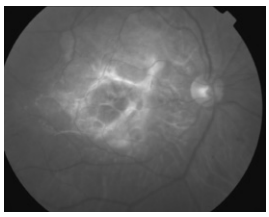

OD


OS

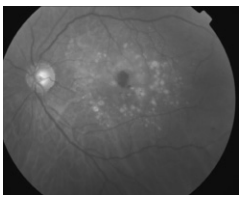
Pre-Operative Data: Macular Translocation Patient_7

Colours / Red Free

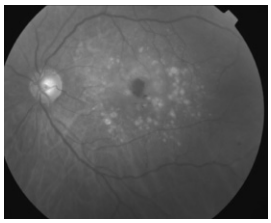

OD

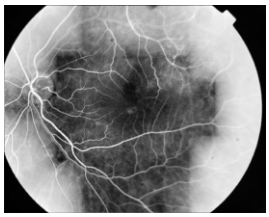


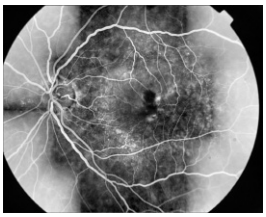

OS

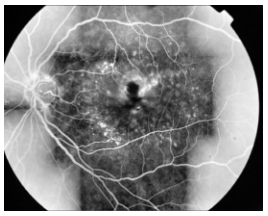


FFA

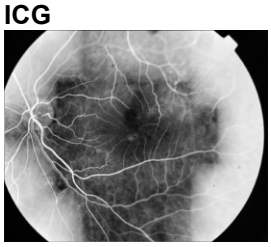

Red Free

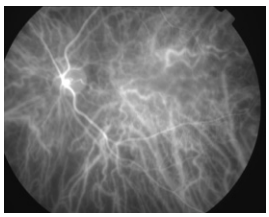

Early

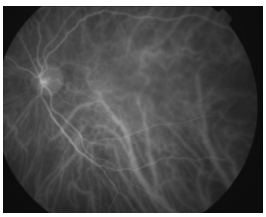

Mid

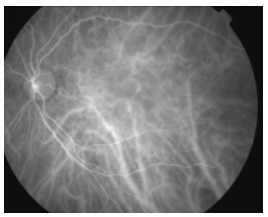

Late

ICG

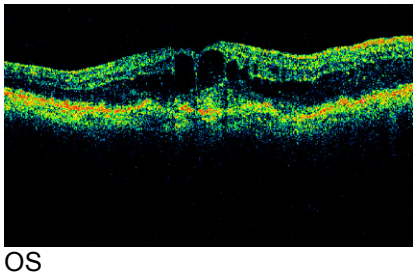

Early FFA

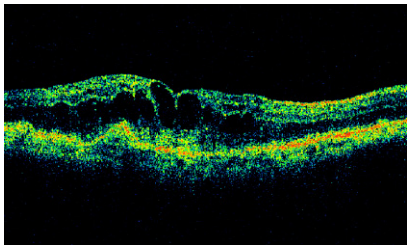

Early


Mid

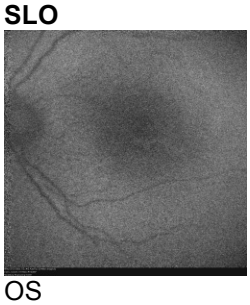

Late

OCT


OS


OS

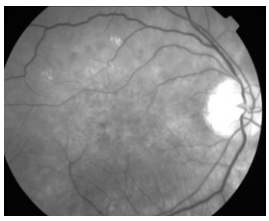
SLO

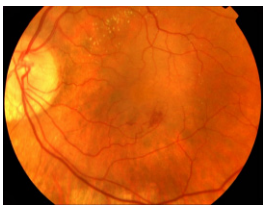

OS

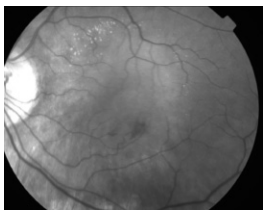
Pre-Operative Data: Macular Translocation Patient_8

Colours / Red Free

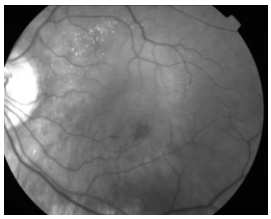

OD

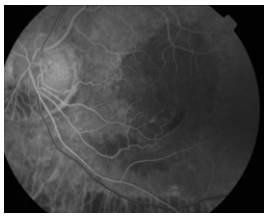


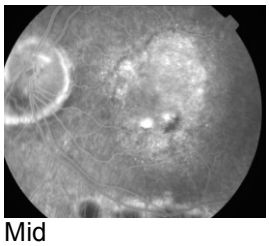

OS

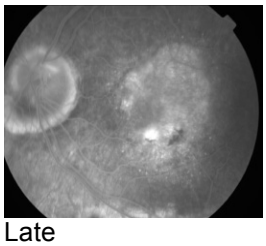


FFA

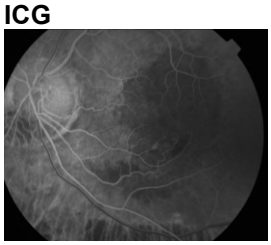

Red Free

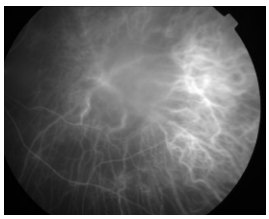

Early

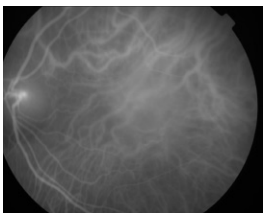

Mid

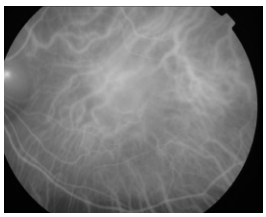

Late

ICG

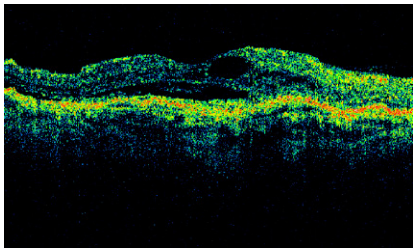

Early FFA

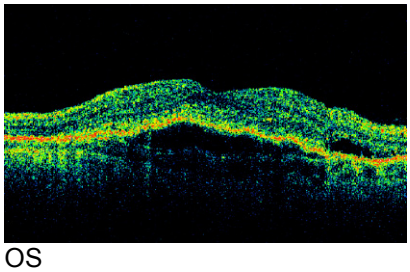

Early

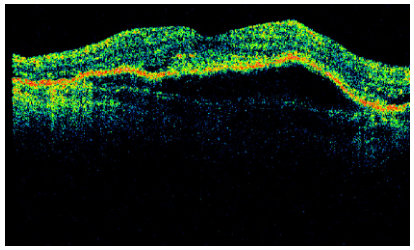

Mid


Late

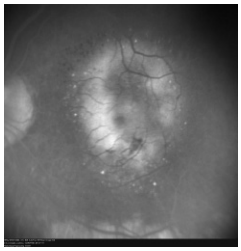
OCT


OD


OS


OS

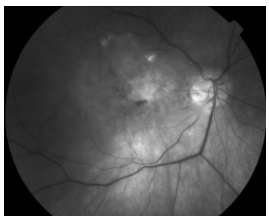
SLO


OD

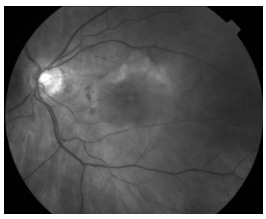
Pre-Operative Data: Macular Translocation Patient_9

Colours / Red Free


OD

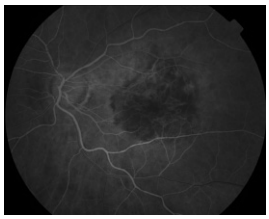


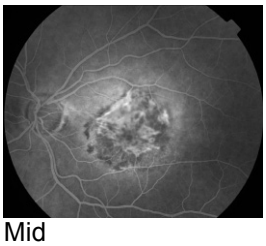

OS

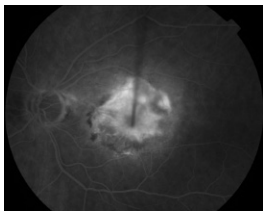


FFA


Red Free


Early


Mid


Late

OCT

OD OS

SLO

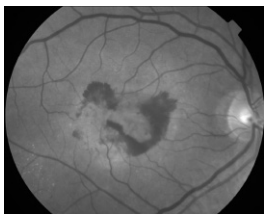

OD

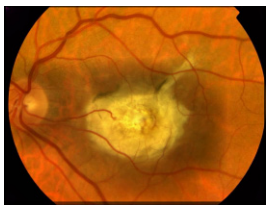
OS

Pre-Operative Data: Macular Translocation Patient_10

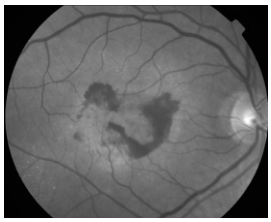
Colours / Red Free


OD




OS

FFA

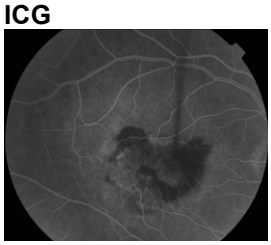

Red Free

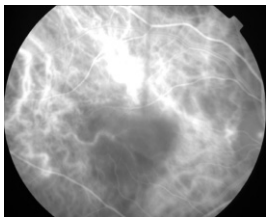

Early

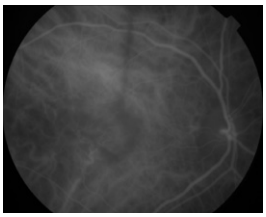

Mid

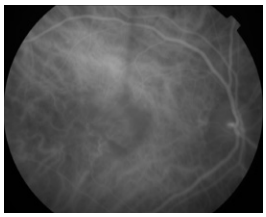

Late

ICG

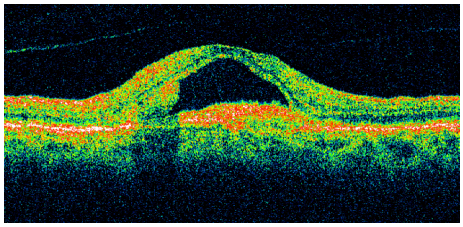

Early FFA


Early

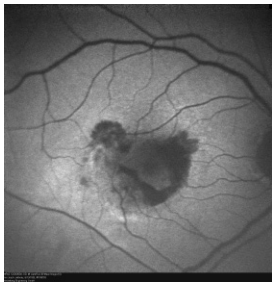

Mid


Late

OCT


OD

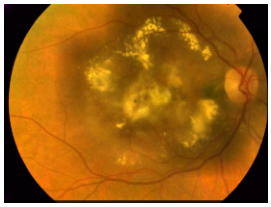
SLO

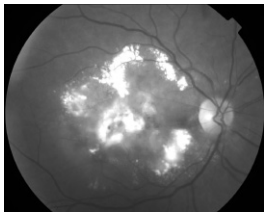

OD

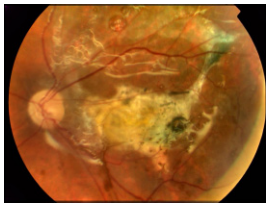
Appendix 6_Macular Translocation: Post-operative Images

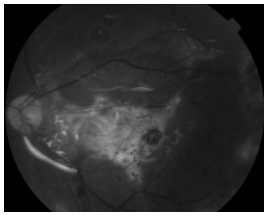
Post-operative Data: Macular Translocation Patient_1

Colours / Red Free

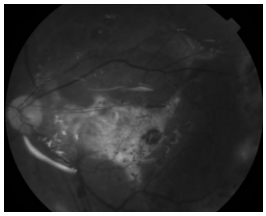

OD

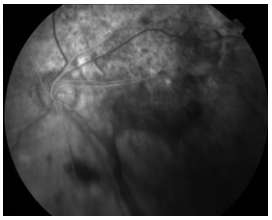


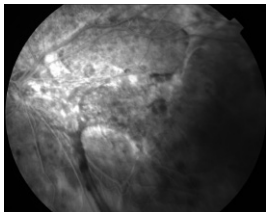

OS

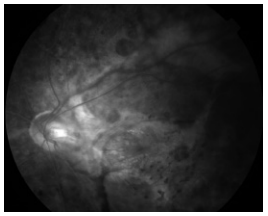


FFA

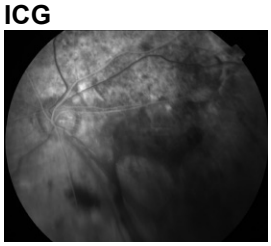

Red Free

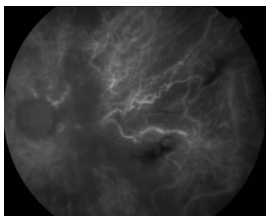

Early

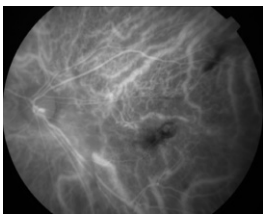

Mid

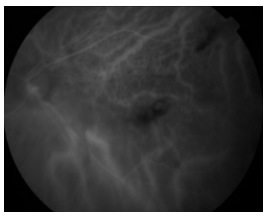

Late

ICG

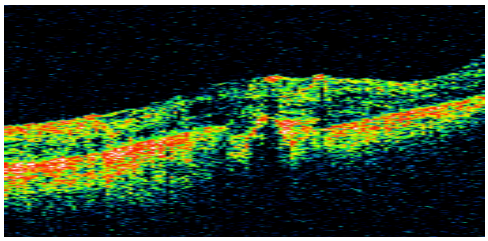

Early FFA


Early


Mid


Late

OCT


OS

Post-operative Data: Macular Translocation Patient_2

Colours / Red Free

OD OS

FFA

Red Free Early Mid Late

ICG

Early FFA Early Mid Late

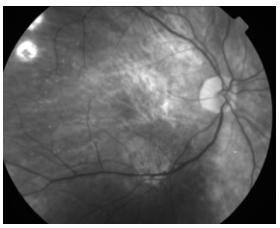
OCT

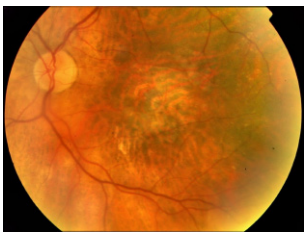
OD OS

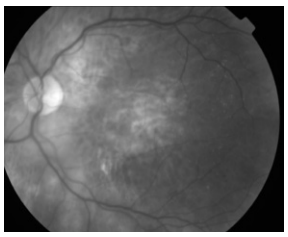
Post-operative Data: Macular Translocation Patient_3

Colours / Red Free

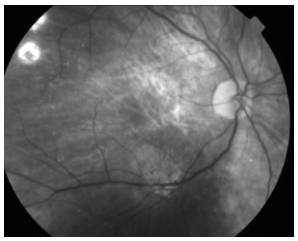

OD

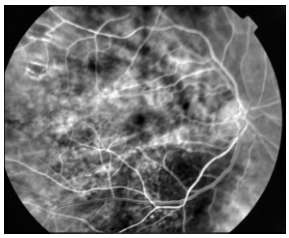


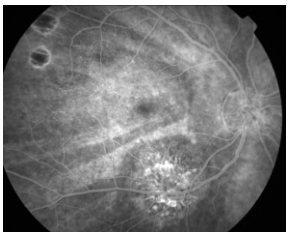

OS

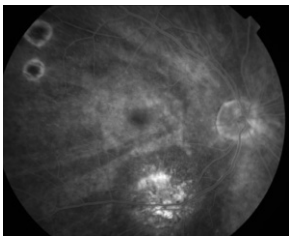


FFA

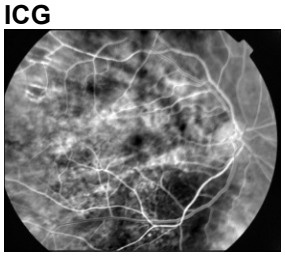

Red Free

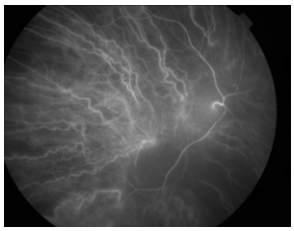

Early

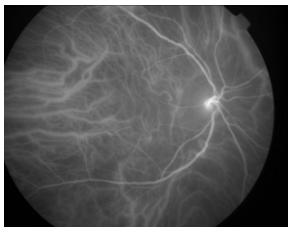

Mid

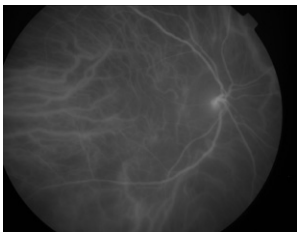

Late

ICG

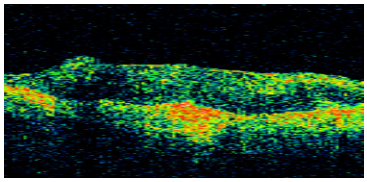

Early FFA


Early

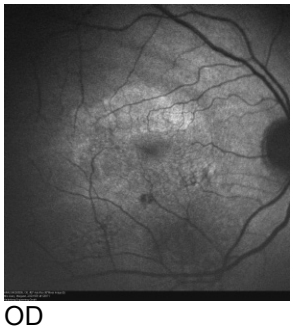

Mid


Late

OCT


OD

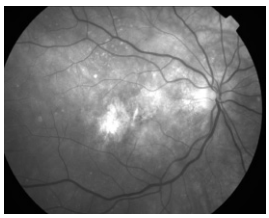
SLO


OD

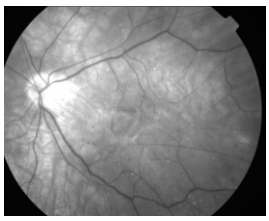
Post-operative Data: Macular Translocation Patient_4

Colours / Red Free

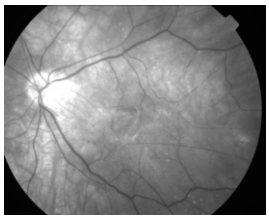

OD

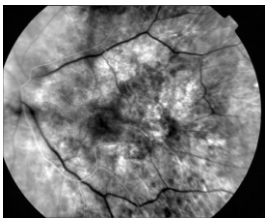


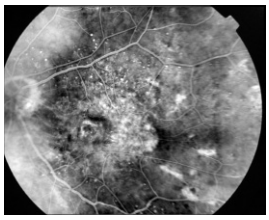

OS

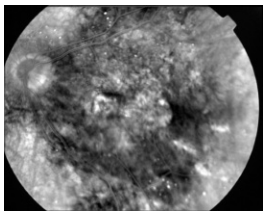


FFA

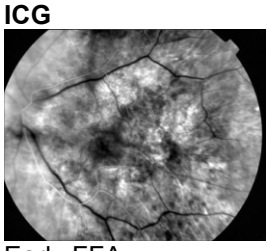


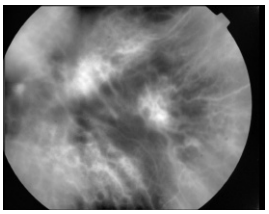

Early

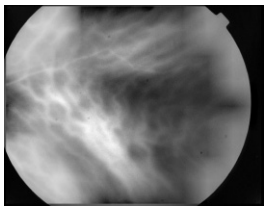

Mid

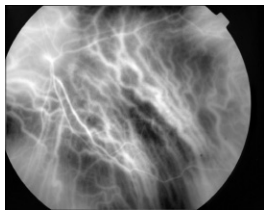

Late

ICG


Early FFA


Early

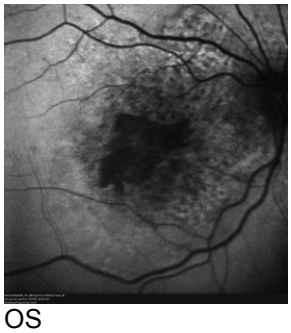

Mid


Late

OCT

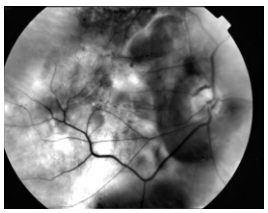
OS

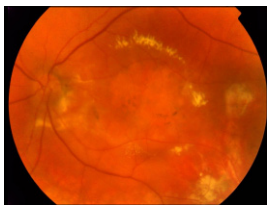
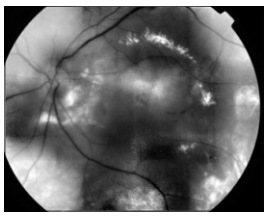
SLO


OS

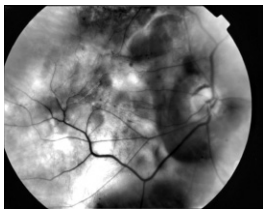
Post-operative Data: Macular Translocation Patient_5

Colours / Red Free



OD



OS

FFA


Red Free

Early Mid Late

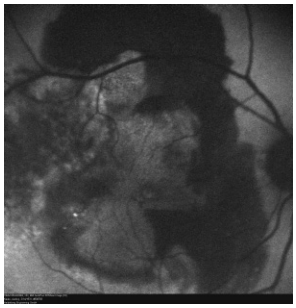
ICG

Early FFA Early Mid Late

OCT

OD

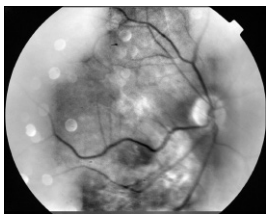
SLO

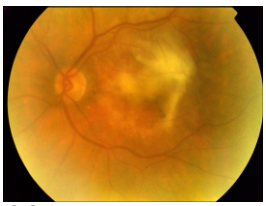

OD

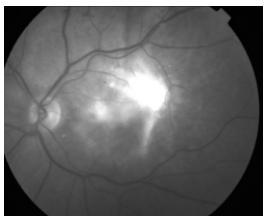
Post-operative Data: Macular Translocation Patient_6

Colours / Red Free

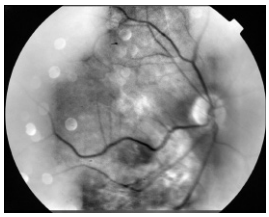

OD

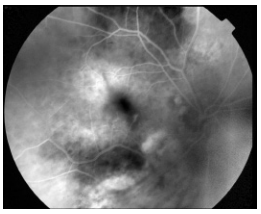


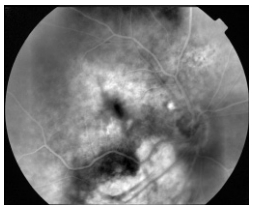

OS

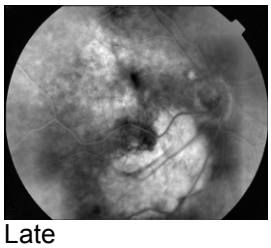


FFA

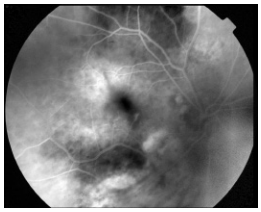

Red Free

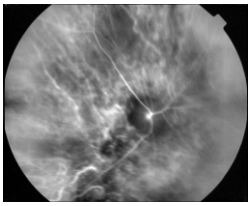

Early


Mid


Late

ICG


Early FFA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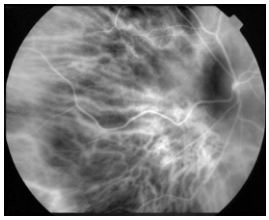
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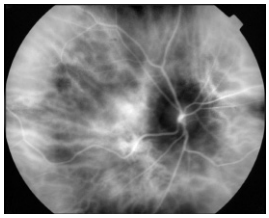
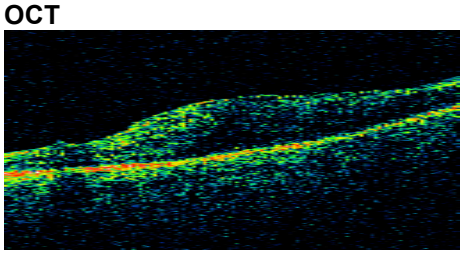
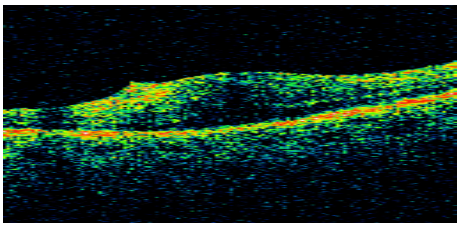

Late

OCT


OD



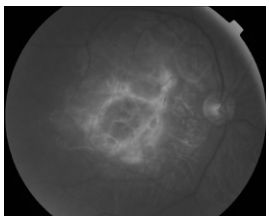
SLO

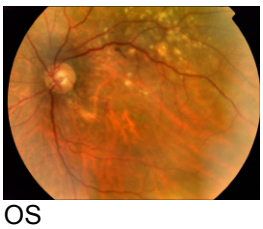
OD OS

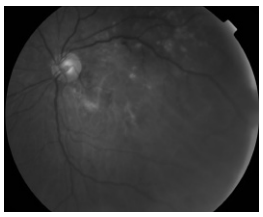
Post-operative Data: Macular Translocation Patient_7

Colours / Red Free


OD

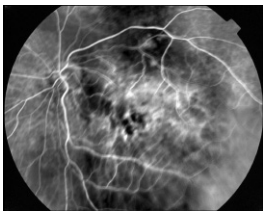


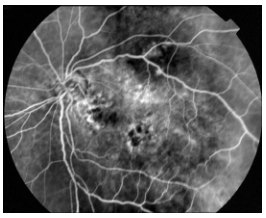

OS

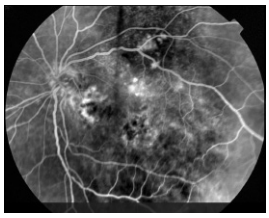


FFA

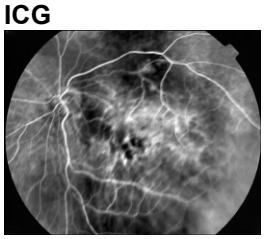

Red Free

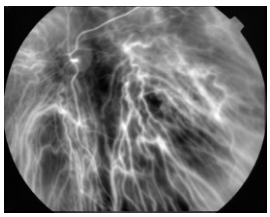

Early


Mid


Late

ICG


Early FFA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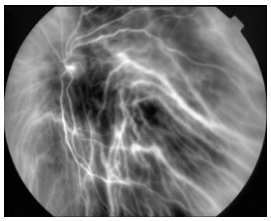
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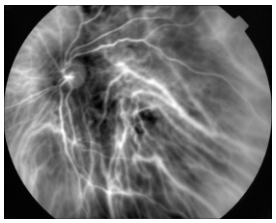
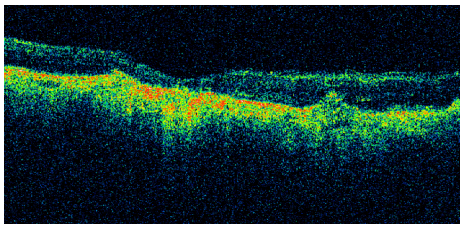

Late

OCT


OS

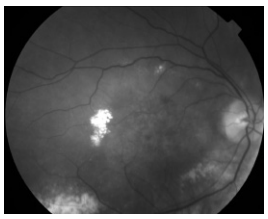
SLO

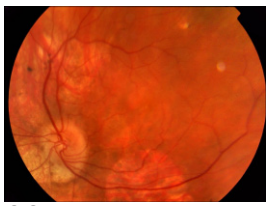

OD

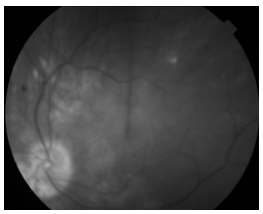
Post-operative Data: Macular Translocation Patient_8

Colours / Red Free

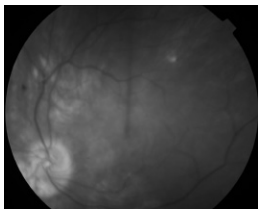

OD

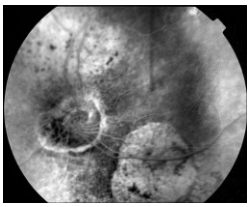


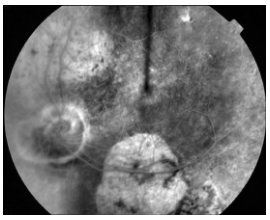

OS

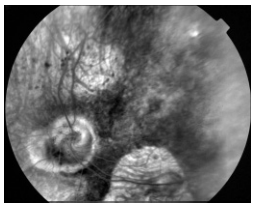


FFA

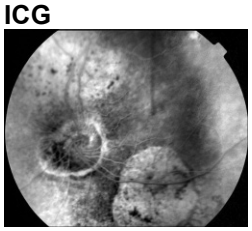

Red Free

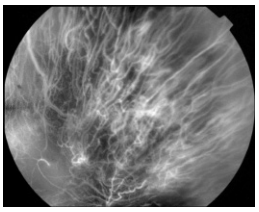

Early

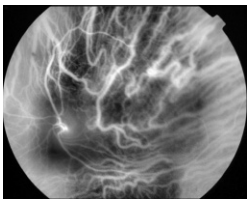

Mid


Late

ICG


Early FFA


Early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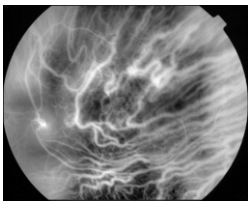

Late

OCT

OS

SLO

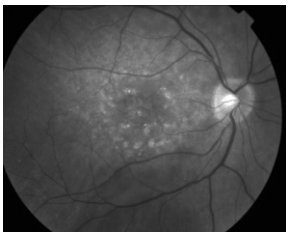
OD OS

Appendix 7_RPE Transplantation: Pre-Operative Images

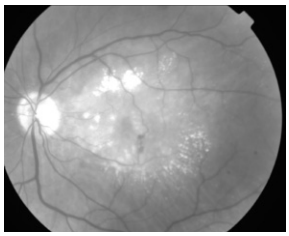
Pre-Operative Data: RPE Translocation Patient_1

Colours / Red Free

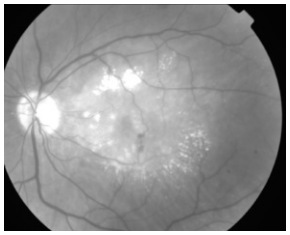

OD




OS

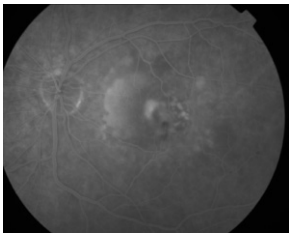


FFA

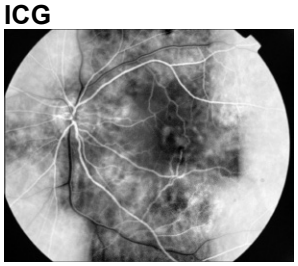

Red Free

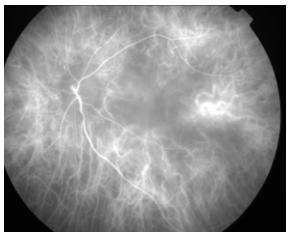

Early

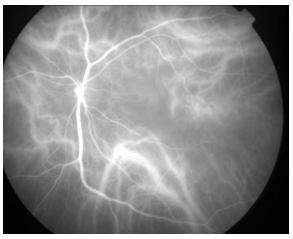

Mid

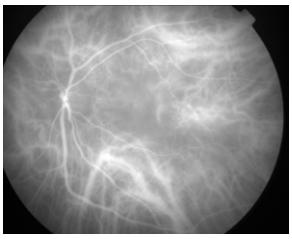

Late

ICG

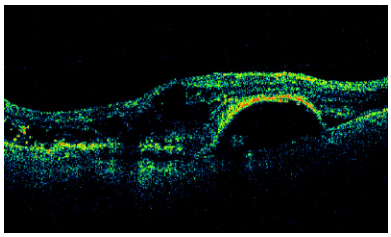

Early FFA

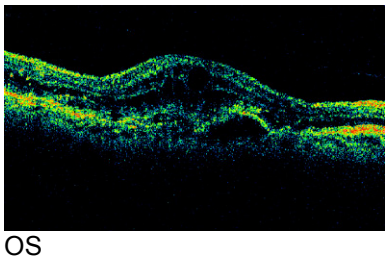

Early


Mid


Late

OCT


OS


OS

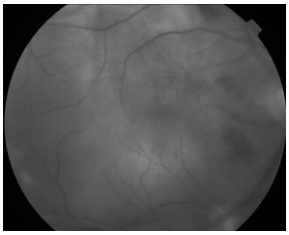
SLO

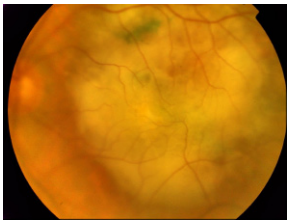

OS

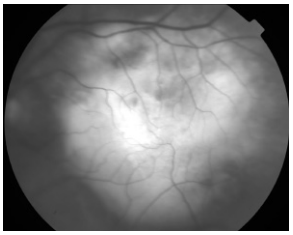
Pre-Operative Data: RPE Transplantation Patient_2

Colours / Red Free

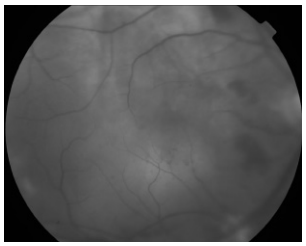

OD

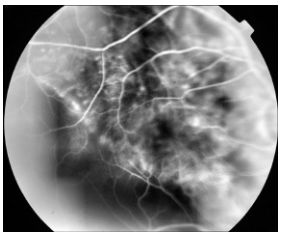


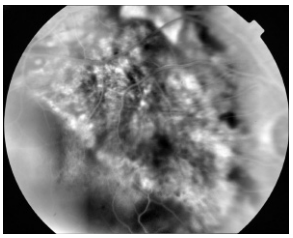

OS



FFA


Red Free


Early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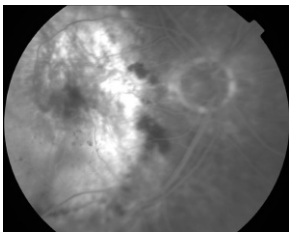
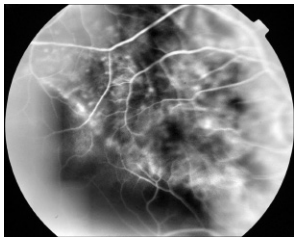
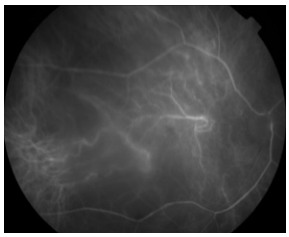
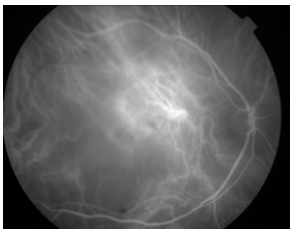

Late

ICG


Early FFA


Early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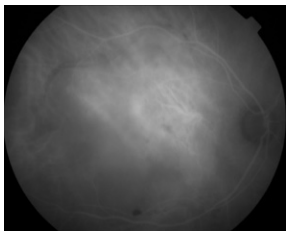
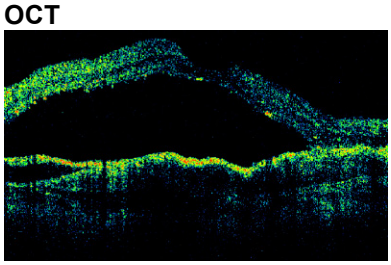
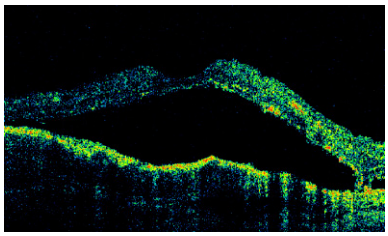

Late

OCT


OD


OD

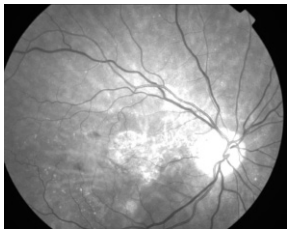
SLO

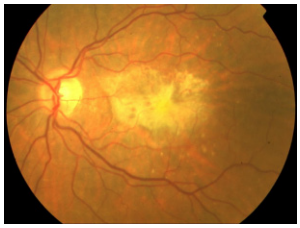

OD

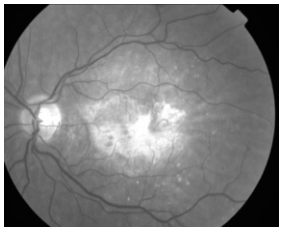
Pre-Operative Data: RPE Transplantation Patient_3

Colours / Red Free

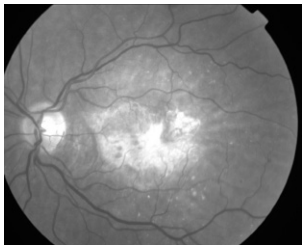

OD

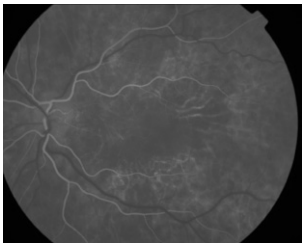


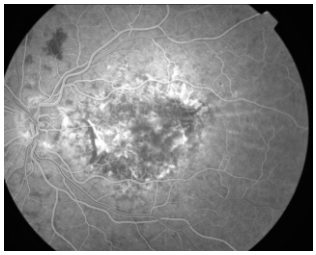

OS



FFA


Red Free


Early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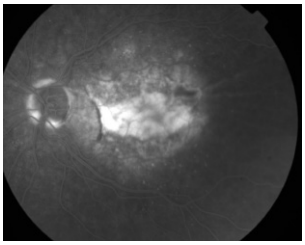
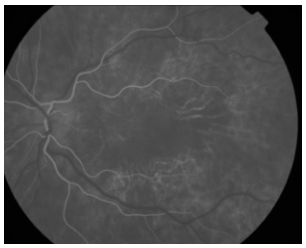
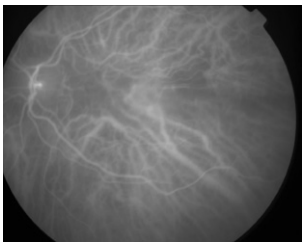
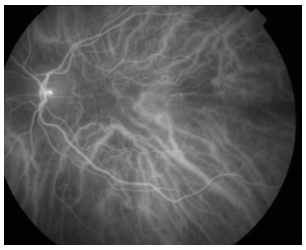
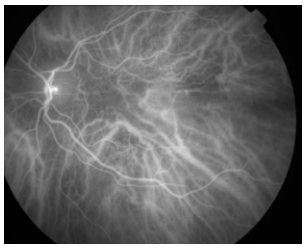

Late

ICG

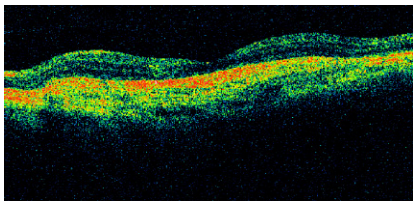

Early FFA


Early

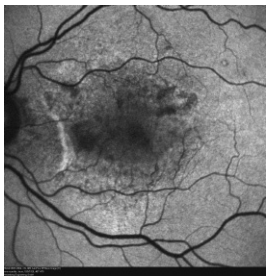

Mid


Late

OCT


OS

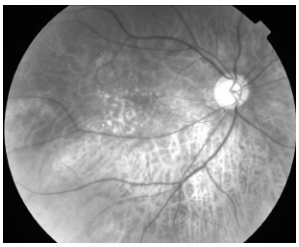
SLO

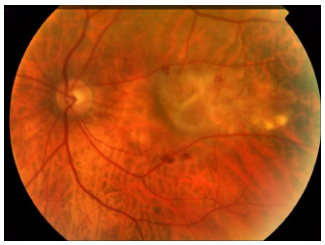

OS

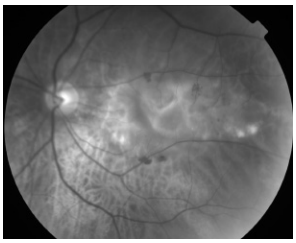
Pre-Operative Data: RPE Transplantation Patient_4

Colours / Red Free

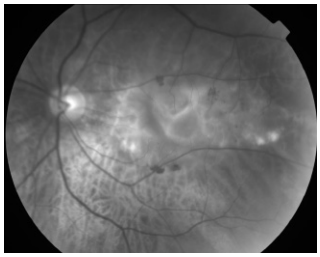

OD

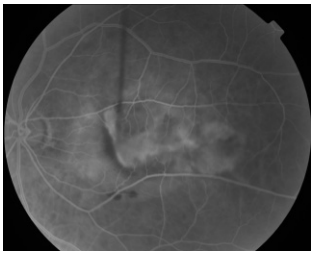


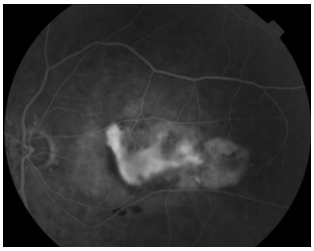

OS



FFA


Red Free


Early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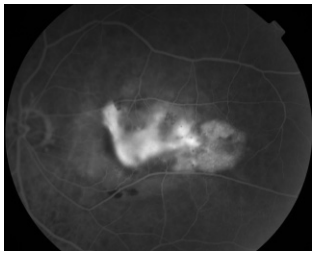
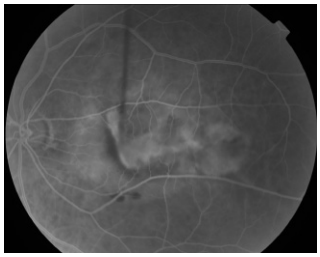
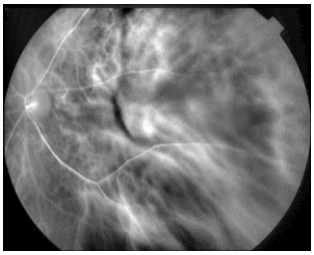
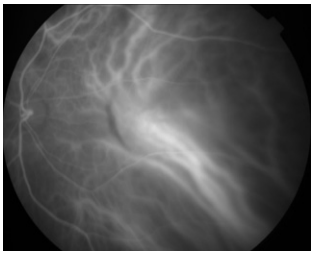
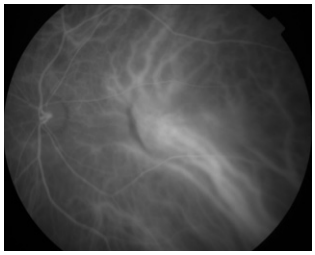

Late

ICG

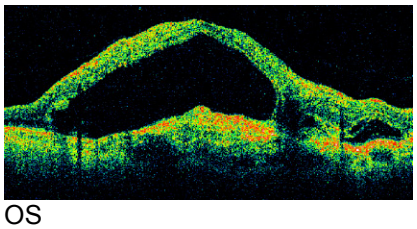

Early FFA

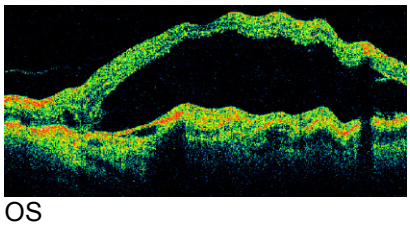

Early


Mid

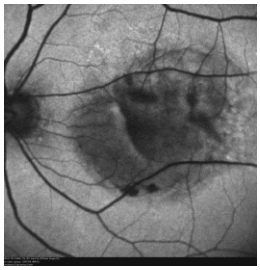

Late

OCT


OS


OS

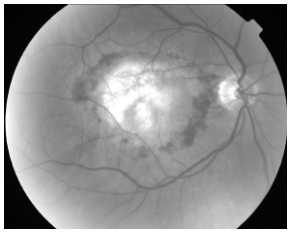
SLO


OS

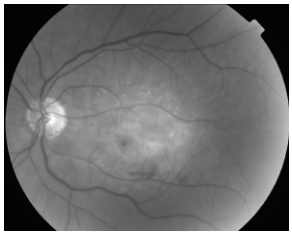
Pre-Operative Data: RPE Transplantation Patient_5

Colours / Red Free

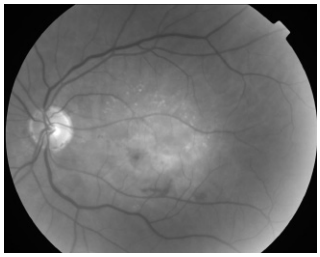

OD

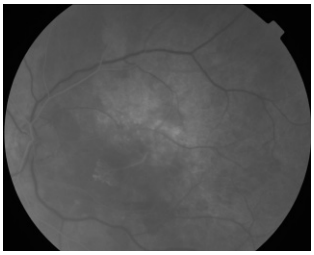


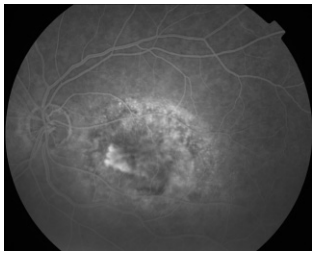

OS

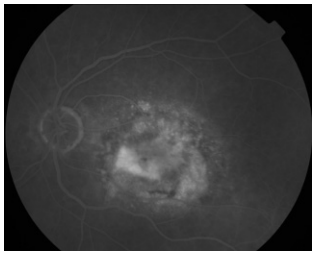


FFA

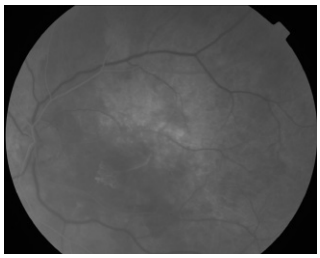

Red Free

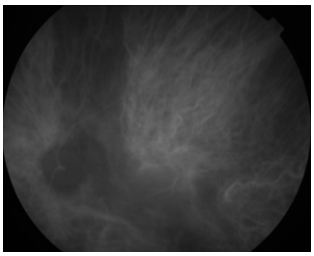

Early

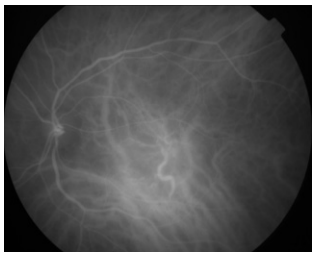

Mid

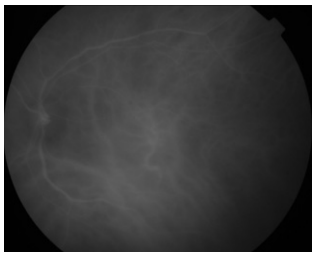

Late

ICG

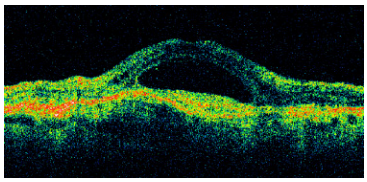

Early FFA

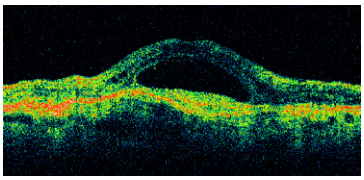

Early


Mid

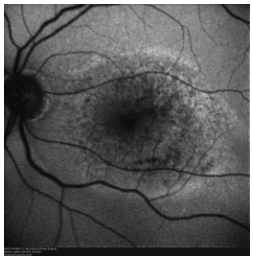

Late

OCT


OS

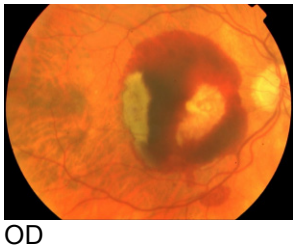

OS

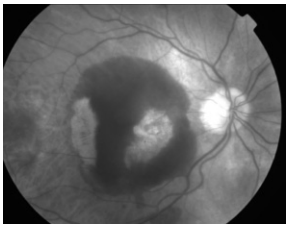
SLO

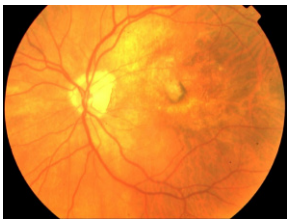

OS

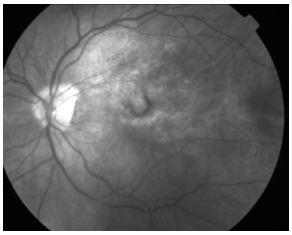
Pre-Operative Data: RPE Transplantation Patient_6

Colours / Red Free


OD

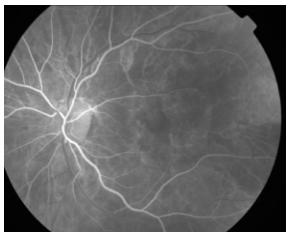


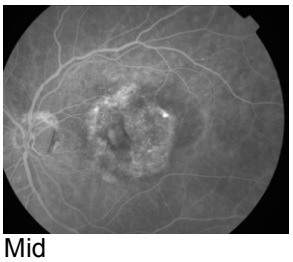

OS

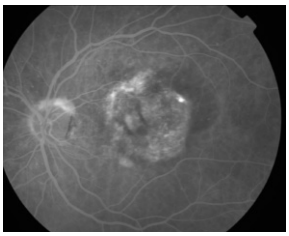


FFA

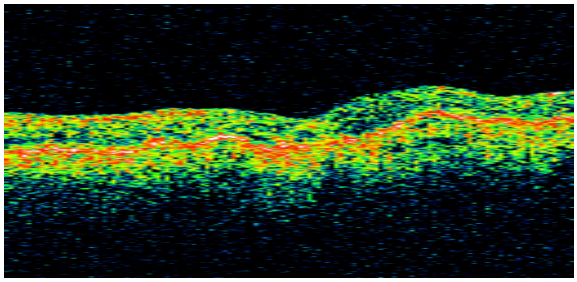

Red Free


Early


Mid


Late

OCT


OS

OS

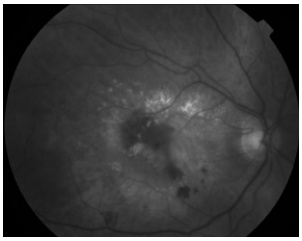
SLO


OS

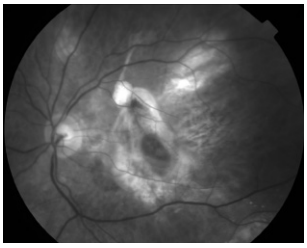
Pre-Operative Data: RPE Transplantation Patient_7

Colours / Red Free

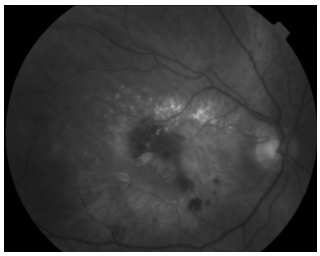

OD

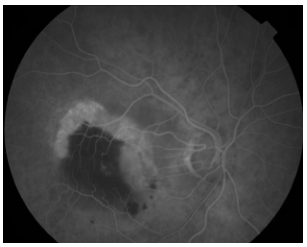


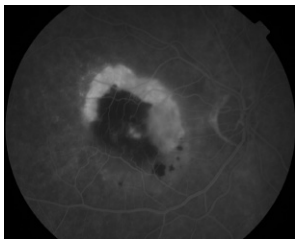

OS

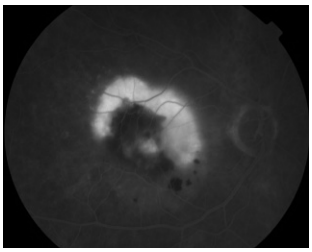


FFA

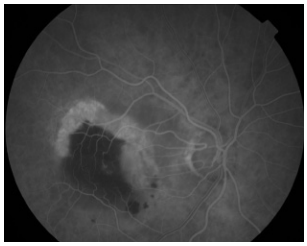

Red Free

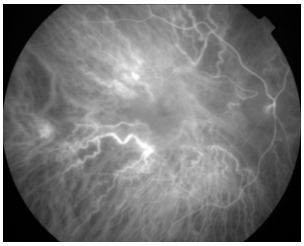

Early

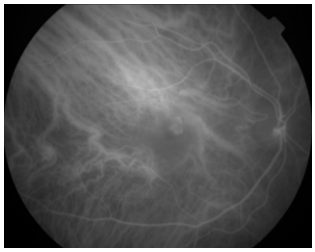

Mid

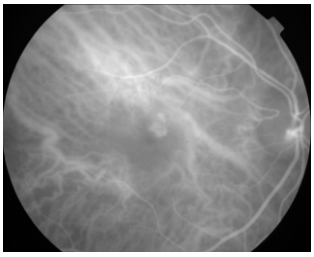

Late

ICG

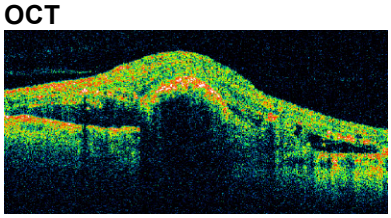

Early FFA

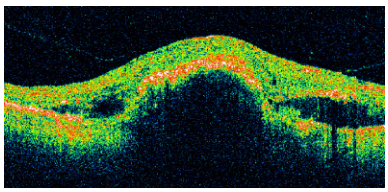

Early


Mid

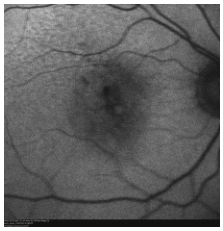

Late

OCT


OD


OD

SLO

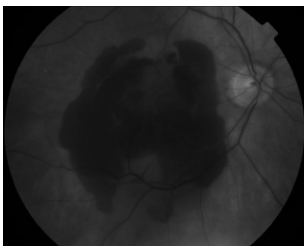

OD

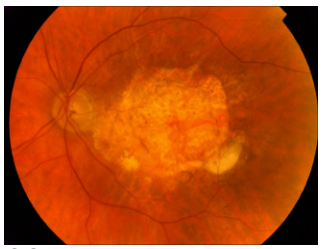

OS

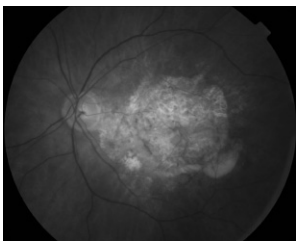
Pre-Operative Data: RPE Transplantation Patient_8

Colours / Red Free

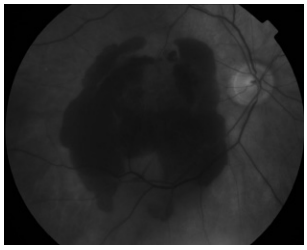

OD

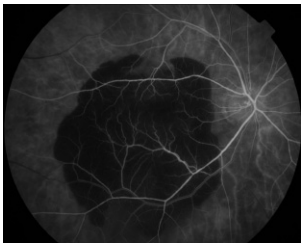



OS

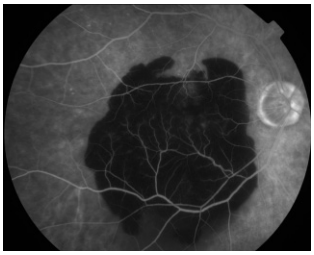


FFA

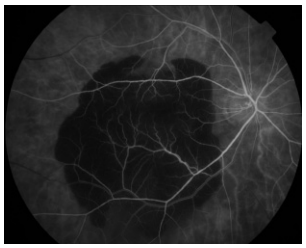

Red Free

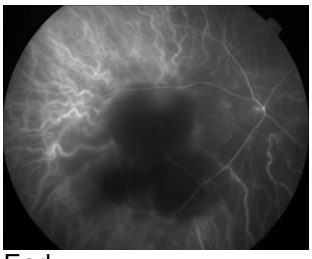

Early

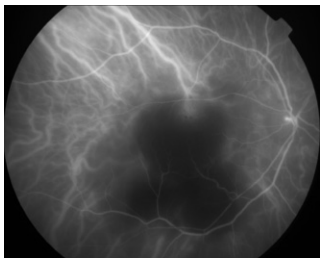

Mid

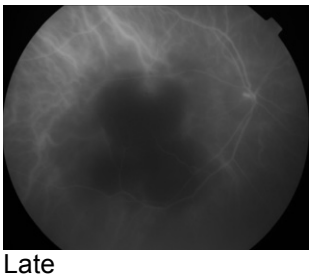

Late

ICG

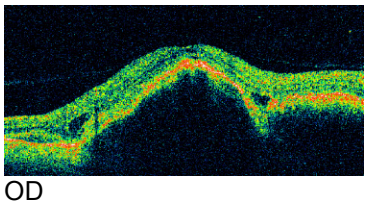

Early FFA


Early

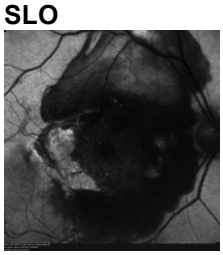

Mid


Late

OCT


OD

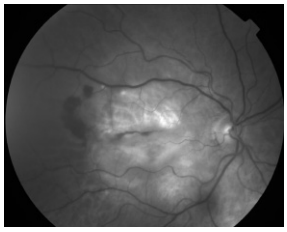
SLO


OD

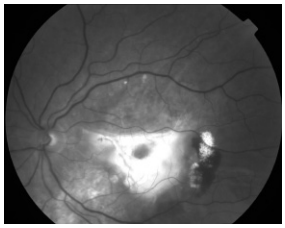
Pre-Operative Data: RPE Transplantation Patient_9

Colours / Red Free

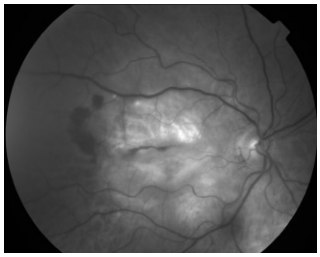

OD

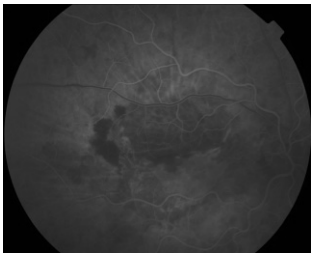


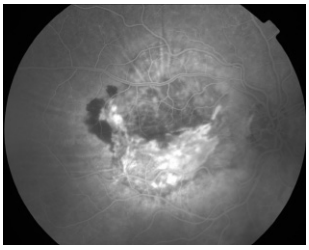

OS

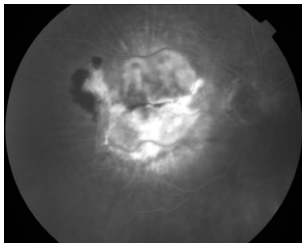


FFA

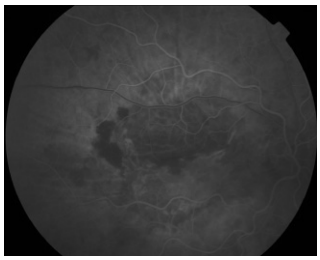

Red Free

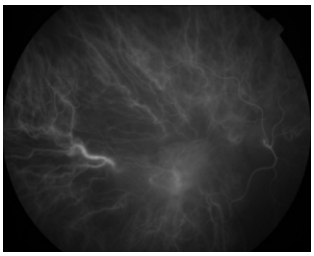

Early

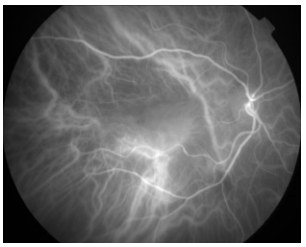

Mid

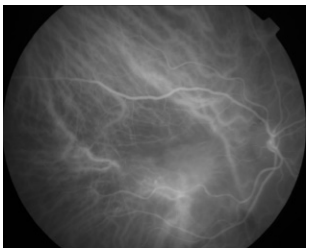

Late

ICG

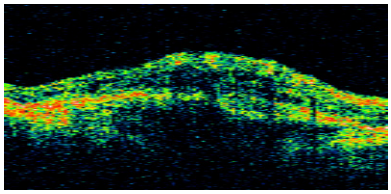

Early FFA

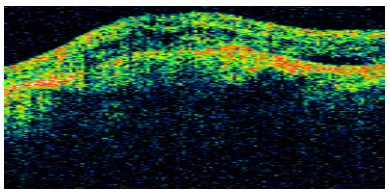

Early


Mid

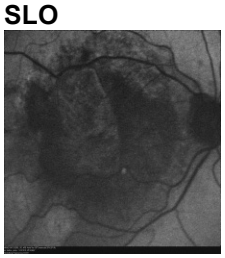

Late

OCT


OD


OD

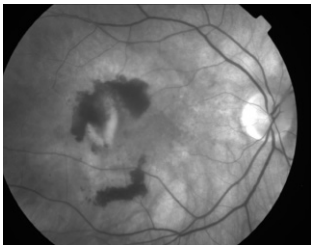
SLO


OD

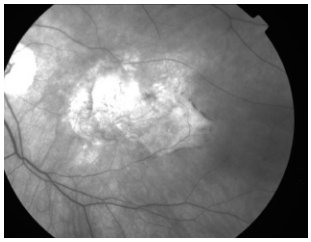
Pre-Operative Data: RPE Transplantation Patient_10

Colours / Red Free

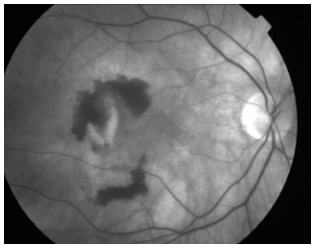

OD

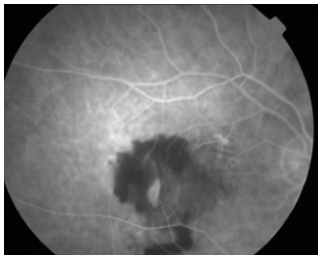


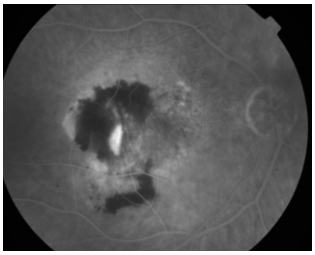

OS

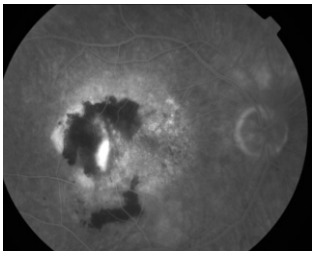


FFA

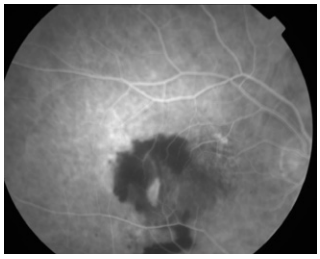

Red Free

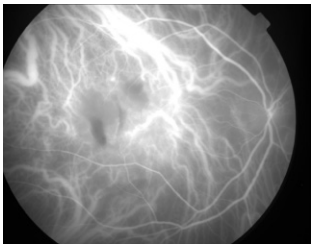

Early

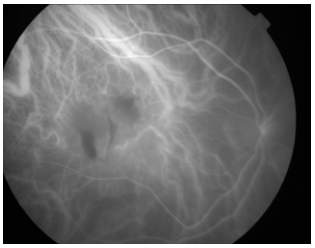

Mid

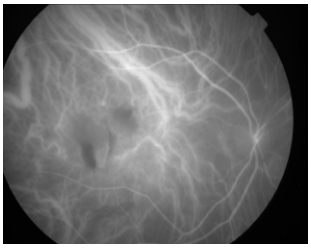

Late

ICG

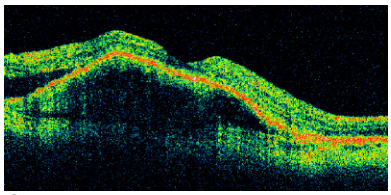

Early FFA

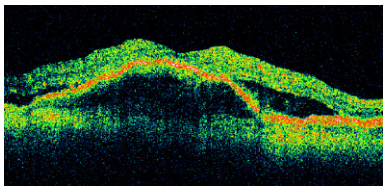

Early


Mid

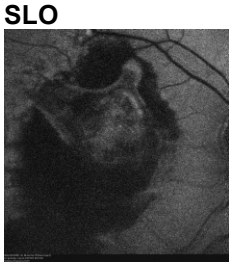

Late

OCT


OD


OD

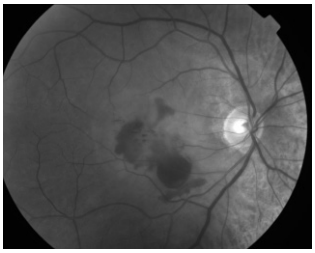
SLO

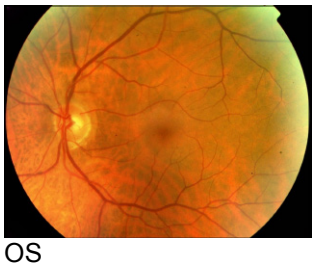

OD

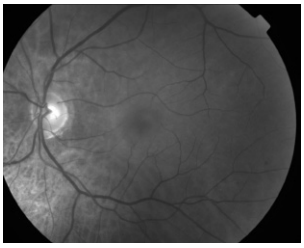
Pre-Operative Data: RPE Transplantation Patient_11

Colours / Red Free

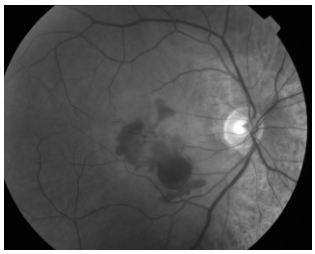

OD

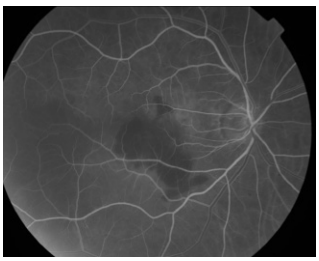



OS

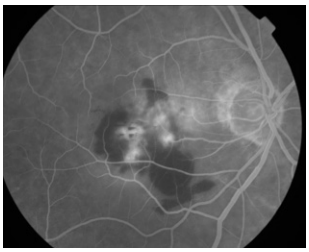


FFA

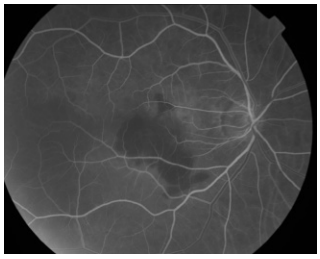

Red Free

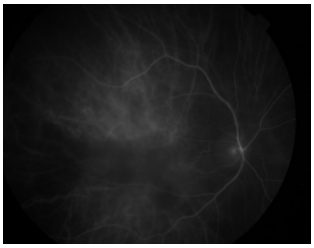

Early

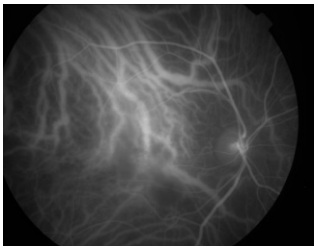

Mid


Late

ICG


Early FFA


Early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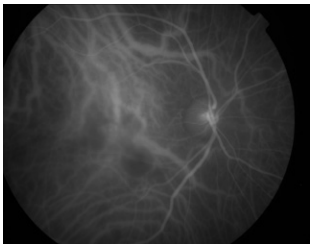
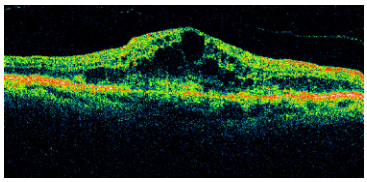

Late

OCT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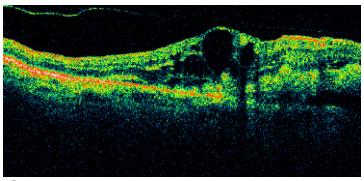
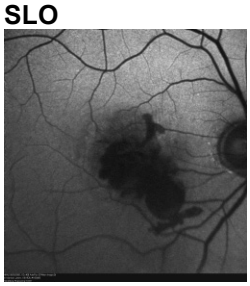

OD

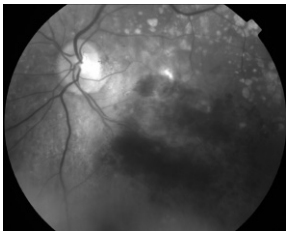
SLO


OD

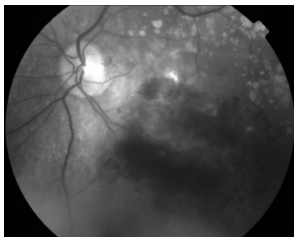
Pre-Operative Data: RPE Transplantation Patient_12

Colours / Red Free

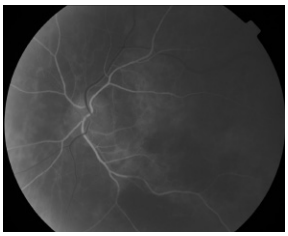
OS



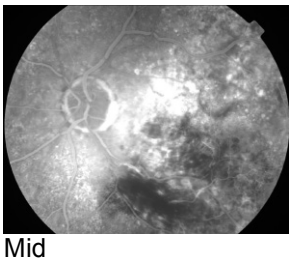
FFA



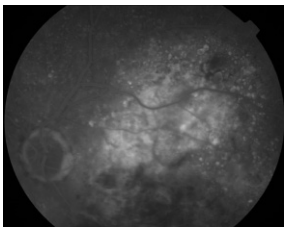
Red Free



Early

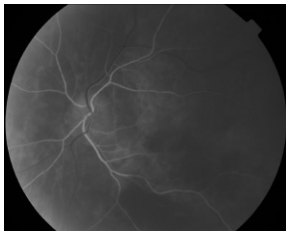


Mid

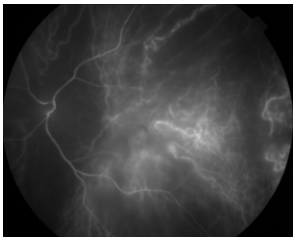


Late

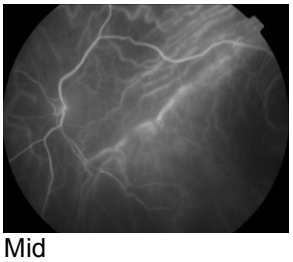
ICG



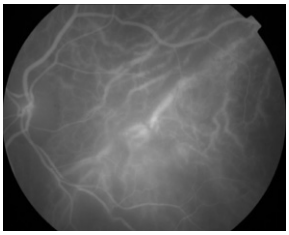
Early FFA



Early

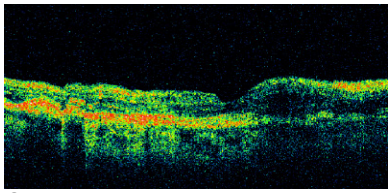


Mid



Late

OCT



OD

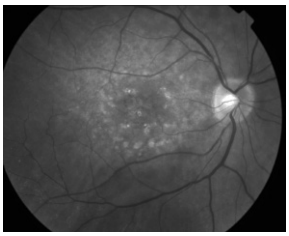
OD

Appendix 8_RPE Transplantation: Post-operative Images

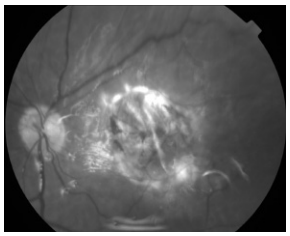
Post-operative Data: RPE Transplantation Patient_1

Colours / Red Free

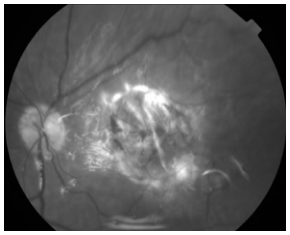
OD



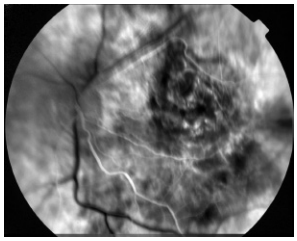
OS



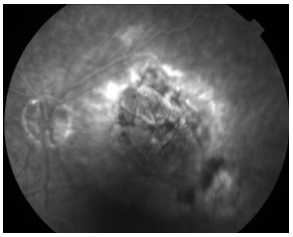
FFA



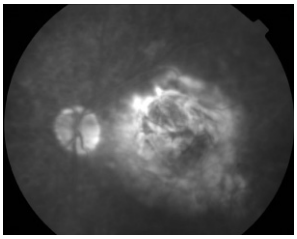
Red Free



Early

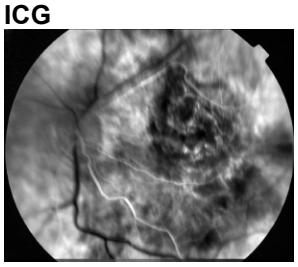


Mid

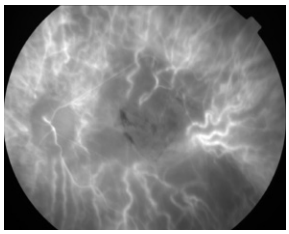


Late

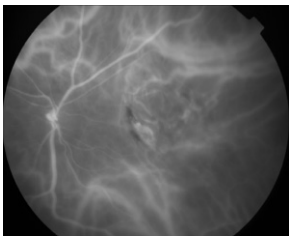
ICG



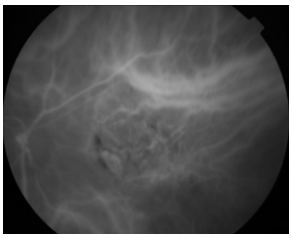
Early FFA



Early

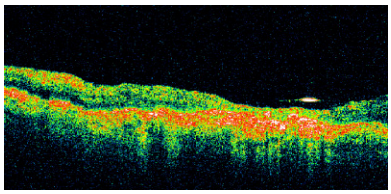


Mid

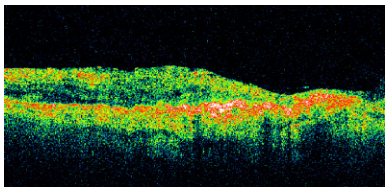


Late

OCT



OS



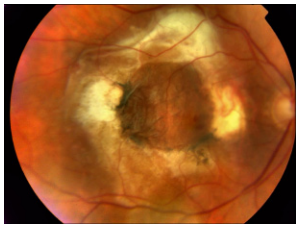
OS

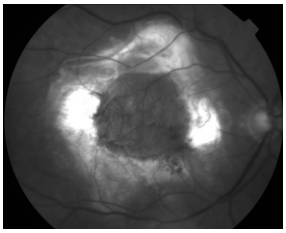
SLO

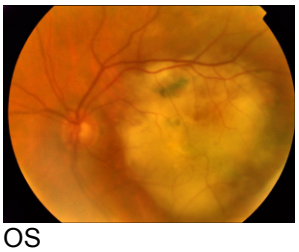
OS

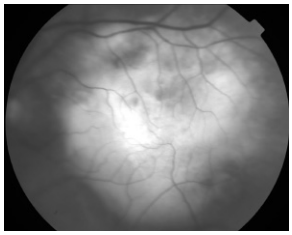
Post-operative Data: RPE Transplantation Patient 2

Colours / Red Free

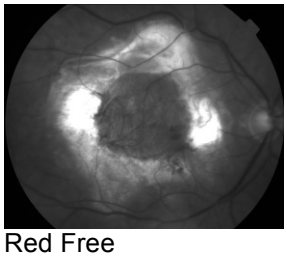

OD

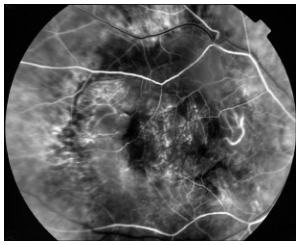


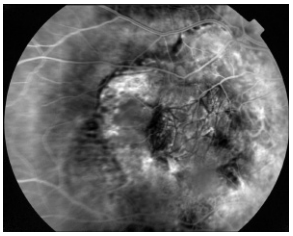

OS



FFA


Red Free


Early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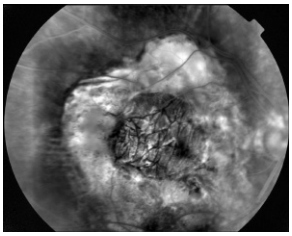
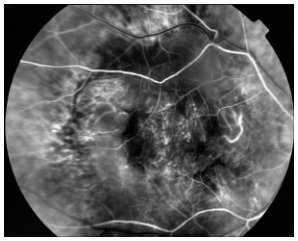
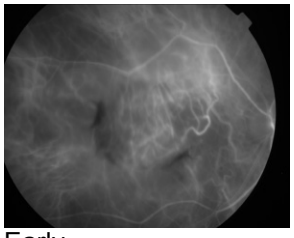
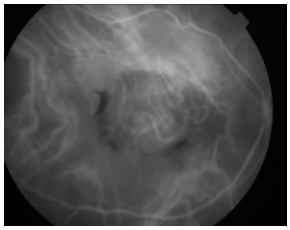
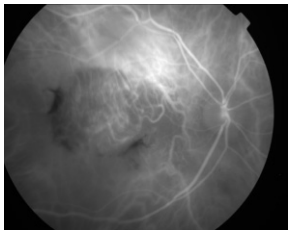

Late

ICG

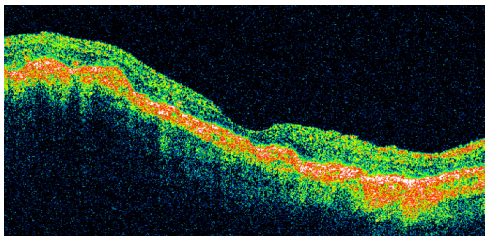

Early FFA

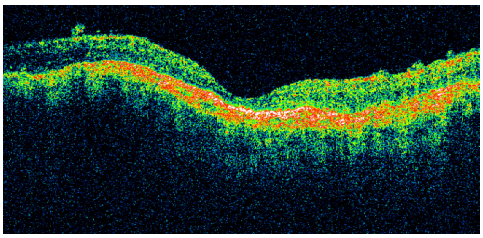

Early


Mid

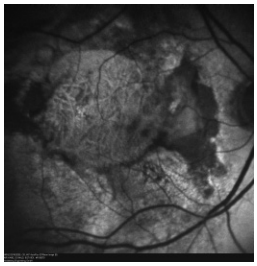

Late

OCT


OD


OD

SLO


OD

Post-operative Data: RPE Transplantation Patient_3

Colours / Red Free

OD OS

FFA

Red Free Early Mid Late

ICG

Early FFA Early Mid Late

OCT

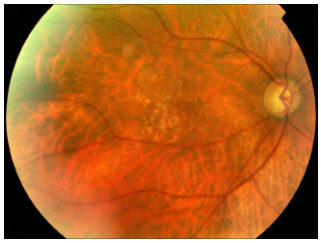
OS OS

SLO

OS

Post-operative Data: RPE Transplantation Patient_4

Colours / Red Free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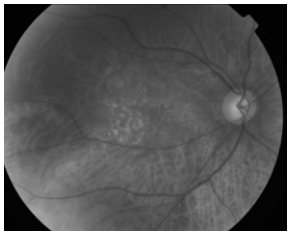
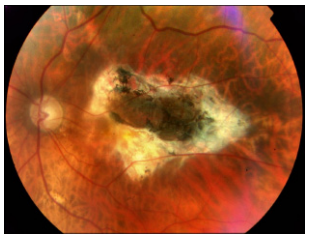
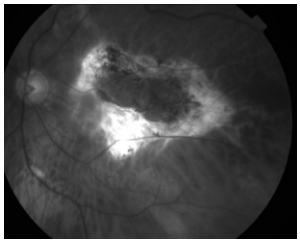
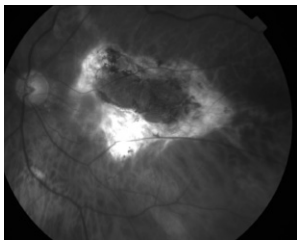
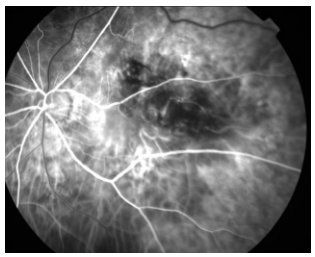


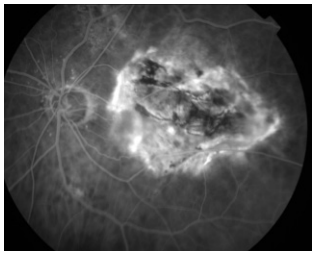

OS

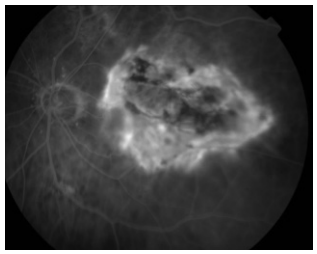


FFA

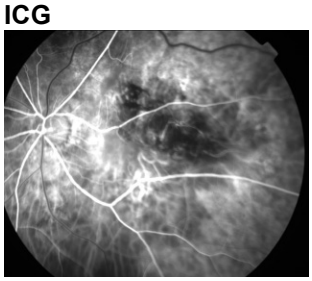

Red Free

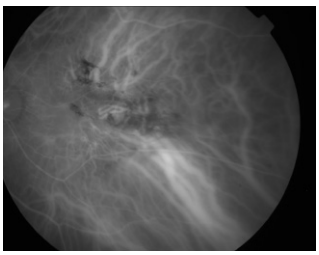

Early

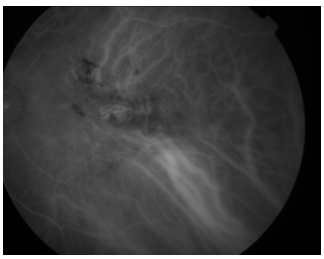

Mid

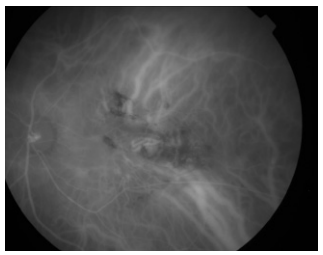

Late

ICG

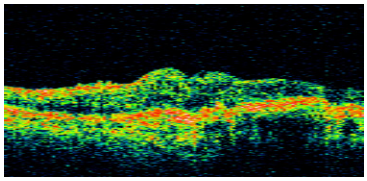

Early FFA


Early

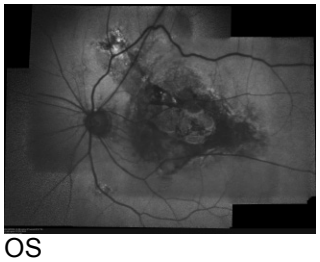

Mid

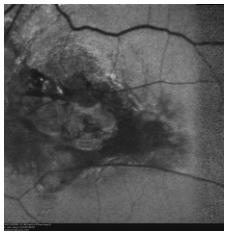

Late

OCT


OS

SLO

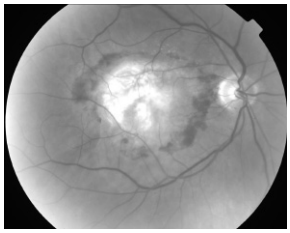


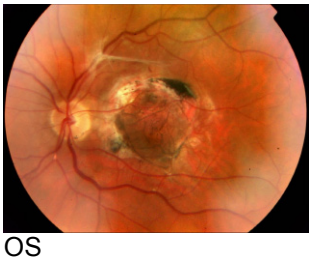

OS

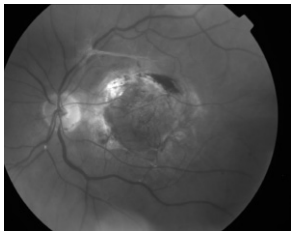
Post-operative Data: RPE Transplantation Patient_5

Colours / Red Free

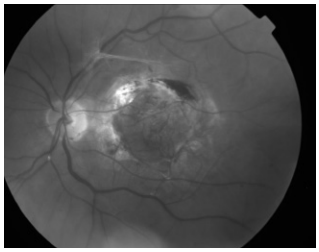

OD

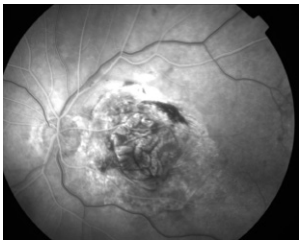


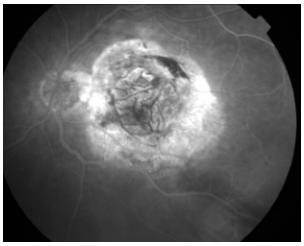

OS



FFA


Red Free


Early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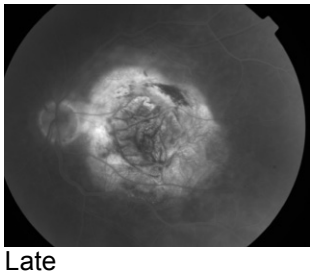
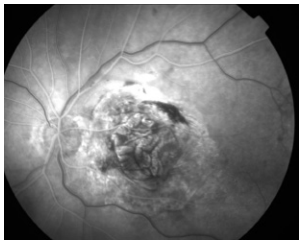
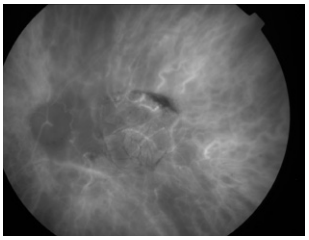

Late

ICG


Early FFA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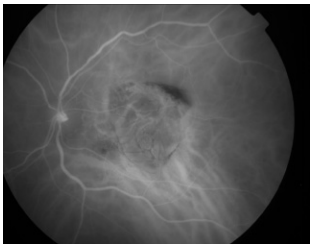
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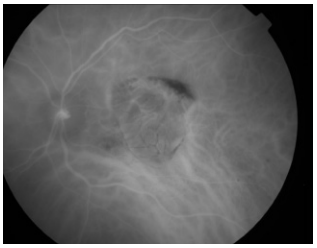
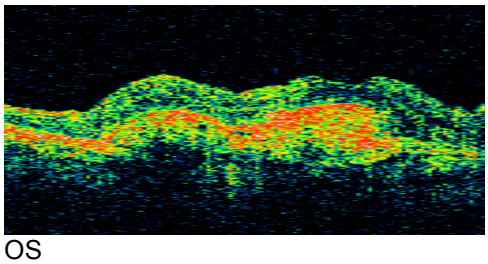

Late

OCT

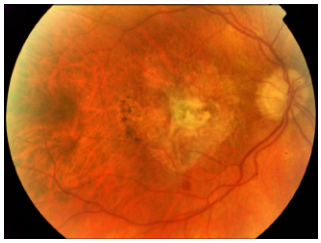

OS

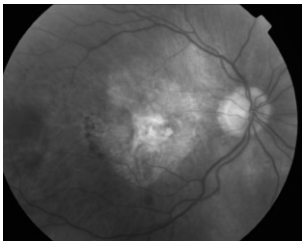
SLO


OS

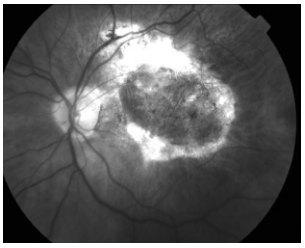
Post-operative Data: RPE Transplantation Patient_6

Colours / Red Free

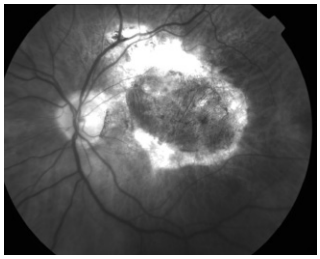

OD

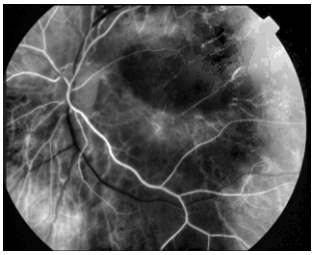


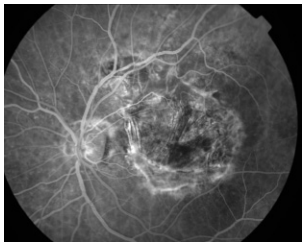

OS



FFA


Red Free


Early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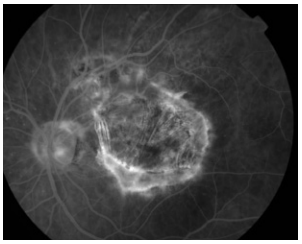
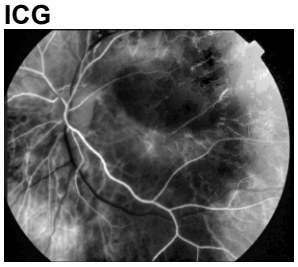
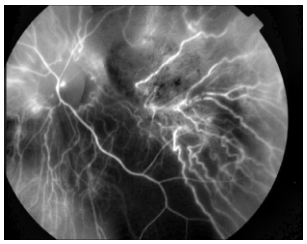
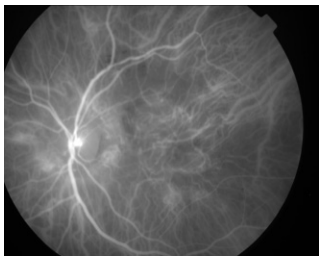

Late

ICG


Early FFA


Early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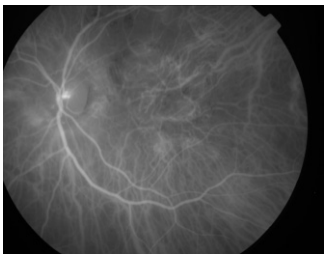
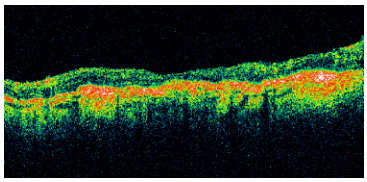
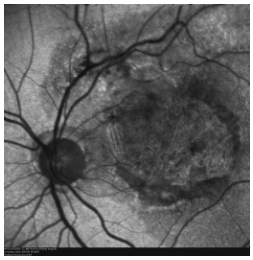

Late

OCT

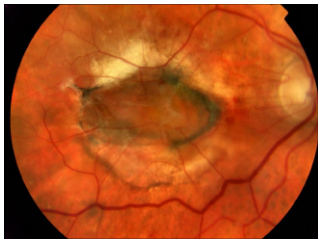

OS

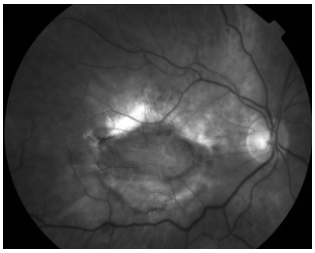
SLO


OS

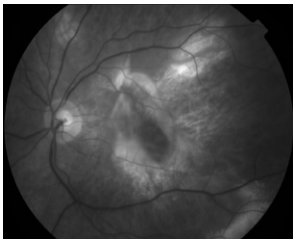
Post-operative Data: RPE Transplantation Patient_7

Colours / Red Free

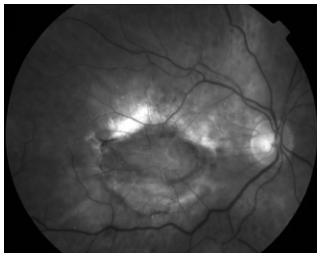

OD

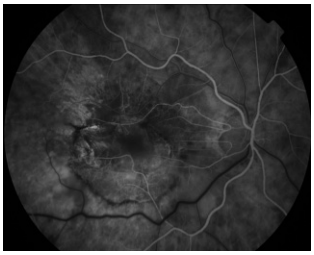


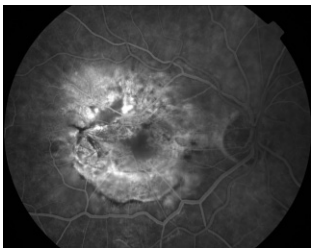

OS



FFA


Red Free


Early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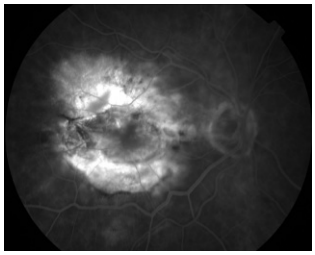
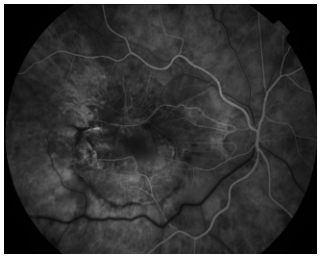
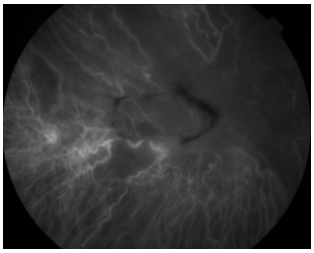
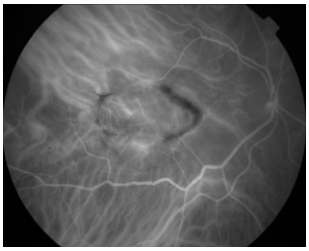
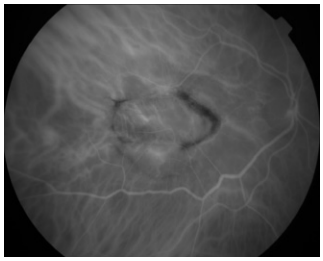

Late

ICG

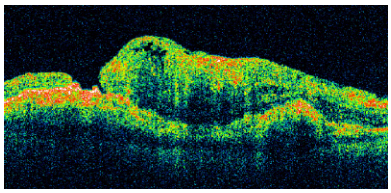

Early FFA


Early


Mid


Late

OCT


OD

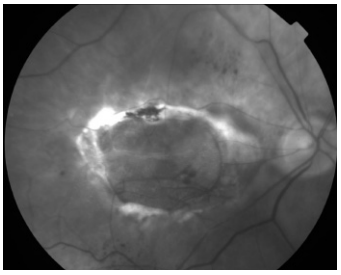
SLO

OD

Post-operative Data: RPE Transplantation Patient_8

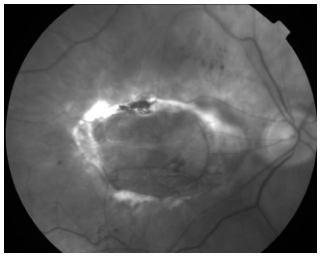
Colours / Red Free

OD

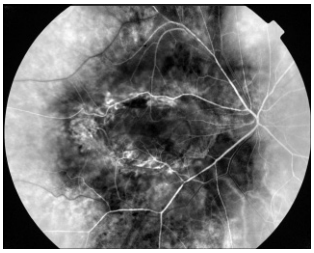


OS

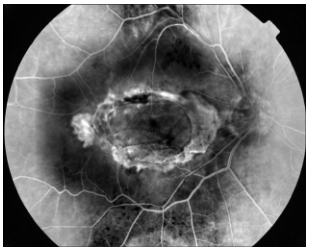
FFA



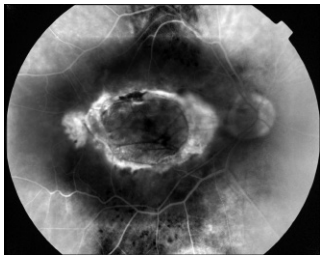
Red Free



Early

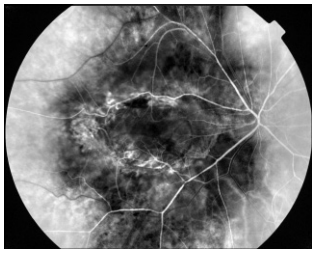


Mid

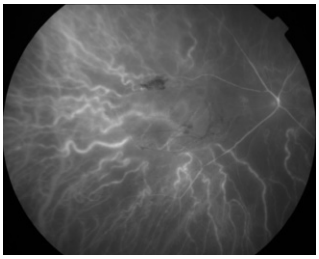


Late

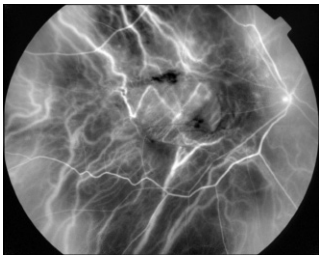
ICG



Early FFA



Early



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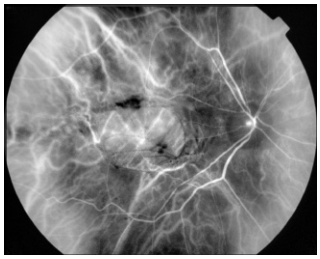
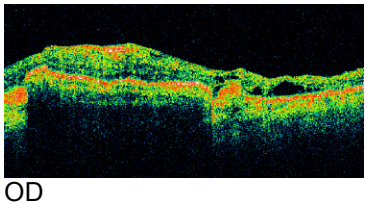


Late

OCT



OD

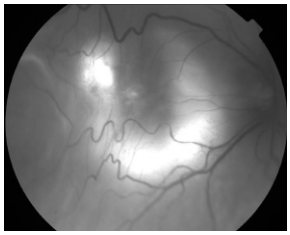
SLO

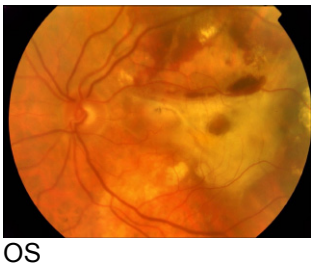
OD

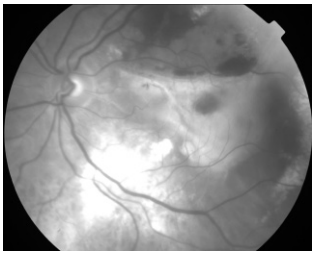
Post-operative Data: RPE Transplantation Patient_9

Colours / Red Free

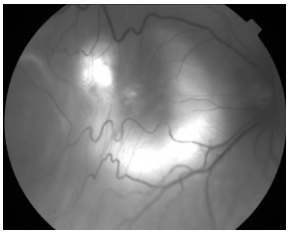

OD

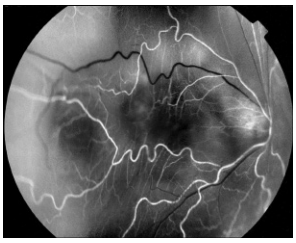


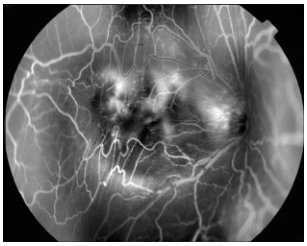

OS



FFA


Red Free


Early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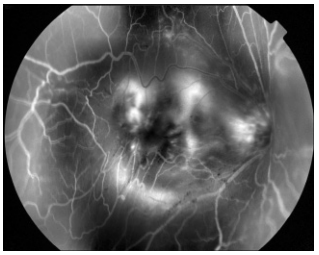
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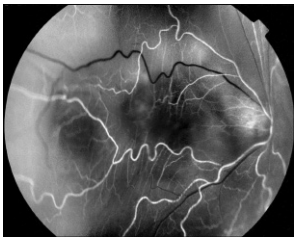
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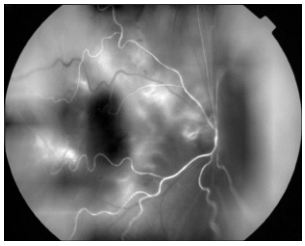
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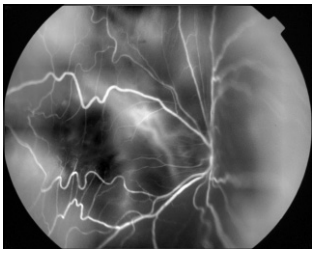
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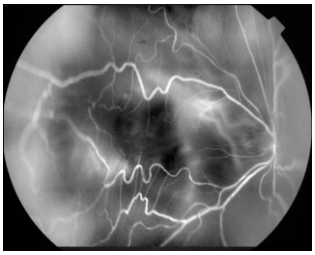
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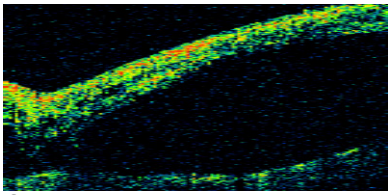
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SLO


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